# STUDIES OF DIETHYLENETRIAMINEPENTAACETIC ACID DEGRADATION IN PULP MILL PROCESS LIQUORS

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Submitted in fulfilment of the requirements for the degree of Master of Science

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November 1998

## DECLARATION

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D. M. Blackwell

November 1998

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# ACKNOWLEDGEMENTS

This project has been developed with the assistance and enthusiasm of many people. Those in the school of chemistry who deserve special thanks include Drs. C. Moorhoff, K. Stack, Brett Paul and A. Grosse. To my supervisors Prof. Paul Haddad and Dr. Des Richardson I extend special thanks for keeping me focused and motivated. I thank Prof. Pierre Krausz (University of Limoges) and Roberto Martinez (National University of Mexico) for samples, methods and spectral data on 2-piperazinone. I would like to thank my fellow post graduate friends for strong social/ work support throughout the course of the project, particularly when energy/ motivation were flagging.

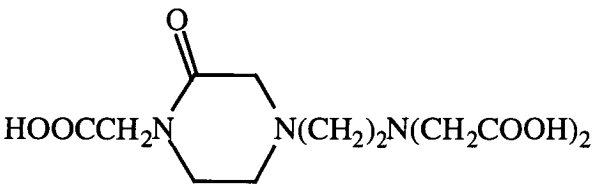
The advice from Roger Brown (Monash University) and Alan Sargeson (ANU) concerning hydrolysis/ esterification of nitriles was also much appreciated. The assistance of Alan Arnold (ADFA) with software and advice for determination of stability constants is gratefully acknowledged.

The CSL staff, involved in tireless processing of samples, deserve many thanks; Noel Davies (MS), Graham Rowbottom (micro analysis/ FTIR) and Evan Peacock (NMR). The creativity of the technical staff in fabricating apparatus is also gratefully acknowledged. I wish to recognise the generous financial and in kind support of ANM (Boyer), particularly library services. Thanks also to Akzo Nobel for providing samples and spectra of several novel aminopolycarboxylic acids.

Finally thanks to my family who have provided unwavering emotional support during my post graduate studies.

# ABSTRACT

The general focus of this study was to synthesise 1-(carboxymethyl)-4-[2-[bis(carboxymethyl)amino]ethyl]-2-oxopiperazine (**1a**), a cyclic degradation product of diethylenetriaminepentaacetic acid (DTPA) present in pulping liquors, and then determine its complexing ability with metal ions of relevance to the paper industry.



**1a**

Three independent but related methods were developed for the preparation of **1a**, although ultimately it was necessary to fully develop only one. In this method, 2-chloroethyliminodiacetonitrile was prepared by Mannich's reaction of 2-chloroethylamine, formaldehyde and potassium cyanide and converted to dimethyl 2-chloroethyliminodiacetate by treatment with gaseous HCl in methanol. Alkylation of 1-methoxycarbonylmethyl-2-oxopiperazine with dimethyl 2-chloroethyliminodiacetate produced 1-(methoxycarbonylmethyl)-4-[2-[bis(methoxycarbonylmethyl)amino]ethyl]-2-oxopiperazine, which when hydrolysed in acidic solution gave the trihydrochloride salt of **1a**. The overall yield of **1a** trihydrochloride (via 1-methoxycarbonylmethyl-2-oxopiperazine) was 20%. Using this new method, sufficient quantities of **1a** were synthesised for both characterisation and complexing studies.

Protonation constants for **1a** were determined using both NMR and potentiometric titration methods and showed good general agreement (Table 1). As indicated, one less $pK_a$ was determined by the NMR model.

*Table 1 Comparison of log protonation constants for 1a at T = 25°C and μ = 0.10M (NaClO4) in aqueous solution by $^1H$ NMR and potentiometry*

| $logK_1$ | $logK_2^H$ | $logK_3^H$ | $logK_4^H$ | ref. |
|---|---|---|---|---|
| | 8.5 | 3.0 | 2.0 | this work (HYPNMR) |
| 10.5 | 8.3 | 3.5 | 2.5 | this work (SQ) |

Stability constants were derived from potentiometric titration data. The stability sequence obtained was Fe(III) > Zn(II) > Hg(II) ≅ Pb(II) > Mn(II) and $logK_{ML}$ values ranged between 12.5 and 16.5. The data confirm the anticipated decrease in stability of **1a** metal complexes compared with those of DTPA. However, it is clear that **1a** possesses quite considerable complexing ability, which has possible implications in terms of pulping and environmental issues.

Premature decomposition of both $H_2O_2$ and DTPA by Mn(II) in the pulping process has been found to be unlikely, since the stability of Mn(II)-**1a** is similar to Mn(II)-DTPA so even if DTPA degrades, protection of $H_2O_2$ via complexation of Mn(II) with **1a** would still occur. Another issue of some concern was the influence of **1a** on removal of heavy metals during wastewater treatment, since DTPA exerts some effect on the precipitation of Zn(II) and Mn(II) at this stage. However, this influence is not anticipated to be very significant, due to the likely low level of **1a** in pulping liquors.

The major outcome of this study is that the use of DTPA as a chelating agent in thermomechanical pulping at Australian Newsprint Mills (ANM) Albury site is appropriate given the significant reduction in complexing power and concentration of DTPA degradation products such as **1a** in pulping liquors. Process changes with respect to DTPA (eg. dosage) would appear to be unnecessary, but a monitoring program for **1a** may need to be implemented.

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# CHAPTER 1

## Introduction and Aims

### 1.1 Introduction

In peroxide bleaching of thermomechanical pulp (TMP), the chelating agent DTPA is used to chelate transition metal ions responsible for the decomposition of peroxide. DTPA is usually the chelant of choice because of its stability and complexing ability at alkaline (9-11) bleaching pH.[1,2] It is standard practice to dose pulp with DTPA and/ or silicate prior to or at the bleaching stage.

Transition metal ions, notably $Fe^{3+}$, $Mn^{2+}$ and $Cu^{2+}$, promote the decomposition of hydrogen peroxide ($H_2O_2$) to water and oxygen. As outlined in Table 1.1, these metals interfere with the formation of the active bleaching species, the perhydroxyl anion ($OOH^-$), causing a lowering of bleaching efficiency.

*Table 1.1 Hydrogen peroxide decomposition*

$$H_2O_2 + M^{2+} \longrightarrow M^{3+} + OH^- + OH^{\cdot}$$

$$H_2O_2 + M^{3+} \longrightarrow M^{2+} + OOH^{\cdot} + H^+$$

$$OOH^{\cdot} \longrightarrow O_2 + H^+$$

$$H^{\cdot} + OH^{\cdot} \longrightarrow H_2O$$

$$H^+ + OH^- \longrightarrow H_2O$$

---

$$2\,H_2O_2 \longrightarrow O_2 + 2\,H_2O$$

Metal ions most commonly associated with bleaching of TMP are $Mn^{2+}$, $Fe^{3+}$, $Cu^{2+}$ and $Cr^{3+}$. Typical concentrations for these metals in wood fibre are given in Table 1.2. Other metal ions, such as $Pb^{2+}$, $Zn^{2+}$ and $Hg^{2+}$ also contribute to the degradation of $H_2O_2$, but are present at far lower concentrations.

*Table 1.2 Typical concentrations of metal ions in wood fibre*[3]

| Metal ions (ppm) | | | |
|---|---|---|---|
| $Mn^{2+}$ > | $Fe^{3+}$ > | $Cu^{2+}$ > | $Cr^{3+}$ |
| | (decreasing decomposition potential) | | |
| 100 | < 5 | < 10 | < 5 |

The effectiveness of DTPA as an agent for binding trace metals has been studied extensively.[4,5] Research has focused on achieving optimal DTPA dosages with respect to other pulp additives (eg silicate, magnesium sulfate), evaluated by gains in brightness. However little attention has been directed toward the fate of DTPA in the pulping system.

Release of DTPA into natural waters by pulp and paper mills has a number of possible environmental implications, including :

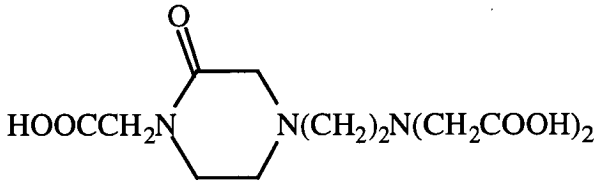
- prevention of heavy metal precipitation and thus removal during effluent treatment
- mobilisation of heavy metals from sediments and other sinks
- ecotoxicity

These concerns have lead to the regulation of its level in plant discharge. In NSW, the operating licence for the ANM Albury paper mill specifies a maximum discharge level for DTPA of 100 ppm (the average concentration of DTPA in

Albury mill effluent between 1991-1997 was 2.4 ppm).[6] In comparison, The National Health and Medical Research Council (NHMRC) have recommended a level of 0.25 ppm ethylenediaminetetraacetic acid (EDTA) in drinking water.[7] There are currently no restrictions on the discharge levels of products from the degradation of DTPA, some of which may also exhibit chelating ability.

## 1.2 Project Aims

Routine monitoring for DTPA at ANM Albury using HPLC has revealed a significant reduction in its level during passage through the pulping system.[8] The disappearance of DTPA from pulp mill process liquor was associated with the appearance of a variety of degradation products, including the recently identified 1-(carboxymethyl)-4-[2-[bis(carboxymethyl)amino]ethyl]-2-oxopiperazine (**1a**). This DTPA breakdown product has also been detected in European river and drinking waters in the upper μg/ L range but only after several enrichment and isolation stages.[9] The study also indicated that **1a** and similar oxopiperazinetricarboxylic acids from the decomposition of DTPA were extremely stable. Other aminopolycarboxylic acid intermediates (eg. EDTA) were detected but unlike **1a** were only transient.




**1a**

*Figure 1.1 Cyclic DTPA degradation product as identified in effluent stream*

Since there is a fair likelihood that **1a** will be a reasonably strong ligand, its chelating ability and concentration in mill discharge are of interest. Thus the two primary objectives of this project were to :

1. synthesise and fully characterise **1a**
2. determine the complexing ability of **1a** with metal ions of relevance to the pulping industry, including iron(III), manganese(II) and mercury(II)

The main purpose of this investigation was to establish the complexing ability of **1a** *relative to* DTPA; the discrete values (quantitative) obtained for stability constants of metal complexes of **1a** were less important than the observed (qualitative) general trend. Provided sufficient evidence was obtained to show the relative difference in complexing ability between DTPA and **1a**, the aims of this project would be satisfied.

### 1.3 Thesis Outline

The project consists of eight chapters, the first of which is the introduction. The remaining seven chapters are organised as follows.

Chapter 2 describes the various processes responsible for the degradation of DTPA in pulping liquors. Degradation mechanisms are explored, products identified and the contribution of each process to DTPA breakdown in papermaking is estimated.

Chapter 3 reviews current methods for the preparation of **1a** type compounds. Possible methods for the preparation of **1a** are then presented and the merits of each identified and discussed.

The methods proposed for synthesis of **1a** are evaluated by experiment in chapter 4.

Techniques for the determination of stability constants of **1a** are discussed in chapter 5. Chapter 6 details the titration work performed to determine stability constants for **1a** and also reports the results of this work.

The experimental details for chromatographic (gas and liquid) method development and all organic preparations are given in chapter 7. Project conclusions and opportunities for future work are presented in chapter 8.

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# CHAPTER 2

## Processes Responsible for Degradation of DTPA in Pulping Liquors

### 2.1 Introduction

As indicated in the previous chapter, the level of DTPA detected in effluent streams is well below that expected, based on the amount dosed and assuming no losses in the process. It is worth devoting some attention to the processes that contribute to the degradation of DTPA, in order to understand and appreciate how **1a** may arise and persist. A variety of processes contribute to the breakdown of DTPA in pulping liquors, including chemical oxidation, photolysis and biological and thermal degradation. Each of these processes will now be discussed.

### 2.2 Possible Mill Locations for Chemical DTPA Degradation

At ANM's Albury mill, DTPA, NaOH and $H_2O_2$ are dosed (in that order) to the secondary refiner as part of the dilution water. After a few seconds retention, bleached pulp is passed to the refined stock tank (RST), where bleaching is quenched by the addition of acid. From the RST pulp moves to the latency chest prior to screening, cleaning and thickening. The temperature gradually declines over these stages and the pulp is dewatered. The average retention time for any given volume of water (and thus DTPA and its degradation products) in the entire pulping circuit (mill-treatment plant) is about 3 days. In contrast, the retention time between DTPA addition and quenching of bleaching is extremely short. It follows then that the opportunity for DTPA breakdown would be greater in the overall circuit, as a result of the approximately 3 day exposure time. A schematic of the Albury TMP plant, showing where DTPA is added, is given in Figure 2.1.

THERMO-MECHANICAL PULPING PLANT

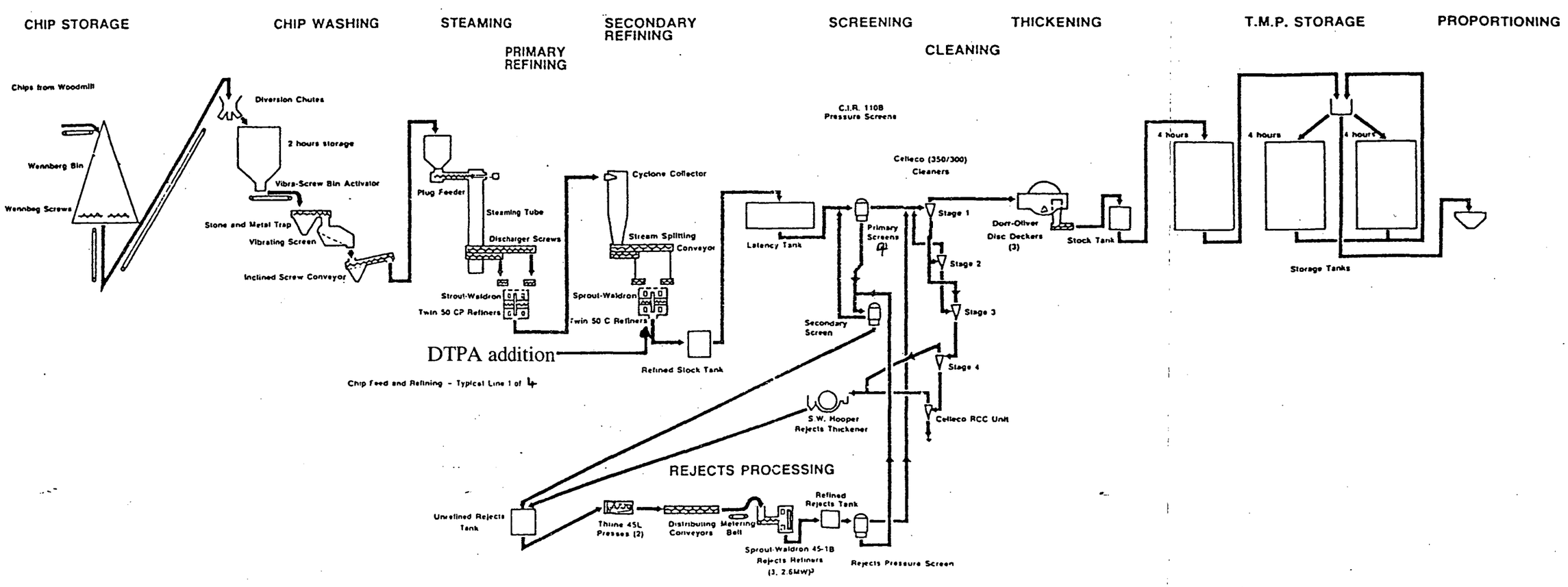


*Figure 2.1 Schematic of the ANM thermomechanical pulping plant*

## 2.3 Chemical Oxidation

Oxidation of EDTA is favoured by elevated temperatures, pH removed from neutral and the presence of metal ions, particularly Mn(II) and Zn(II).[1,2] In pulping, such conditions prevail during the bleaching step. However, DTPA and peroxide are only in contact for a few seconds, so either the kinetics of the reaction are extremely rapid or oxidation is negligible despite favourable conditions. A preference by $H_2O_2$ for destruction of chromophores rather than DTPA is expected during refining, due to high pulp consistency. After quenching of the bleach liquor in the RST, there exists little or no further potential for DTPA oxidation by peroxide. However, oxidation by other means may occur. For example, oxidation of DTPA by Mn(II) at intermediate pH has been observed.[3] Oxidation of DTPA by other metal ions, including Fe(III) and Cu(II) at pH 5.5, is possible depending on the oxidation potentials of these metals and their concentration.

Whilst a number of studies describe the disposition of "free" DTPA toward chemical oxidation,[4,5] similar oxidation studies using EDTA have found a significant reduction in oxidation rate when the ligand was complexed with a metal cation.[5] In pulping liquors one could expect DTPA to exist in both bound and free form; DTPA complexed with metal ions being less amenable to oxidative attack. Nevertheless, whether free or bound there exists the opportunity for oxidation of DTPA in the overall water circuit of the pulp and paper mill.
In an oxidative study of particular interest to the current project,[6] EDTA was successively decarboxylated by $Ce^{4+}$ in acidic media to furnish a cyclic by-product (**5**) which bears strong resemblance to **1a** (Figure 2.2). Compound **5** was stable to further oxidation.

The study provides some important clues as to the mechanism by which DTPA may be degraded chemically in pulping liquors.

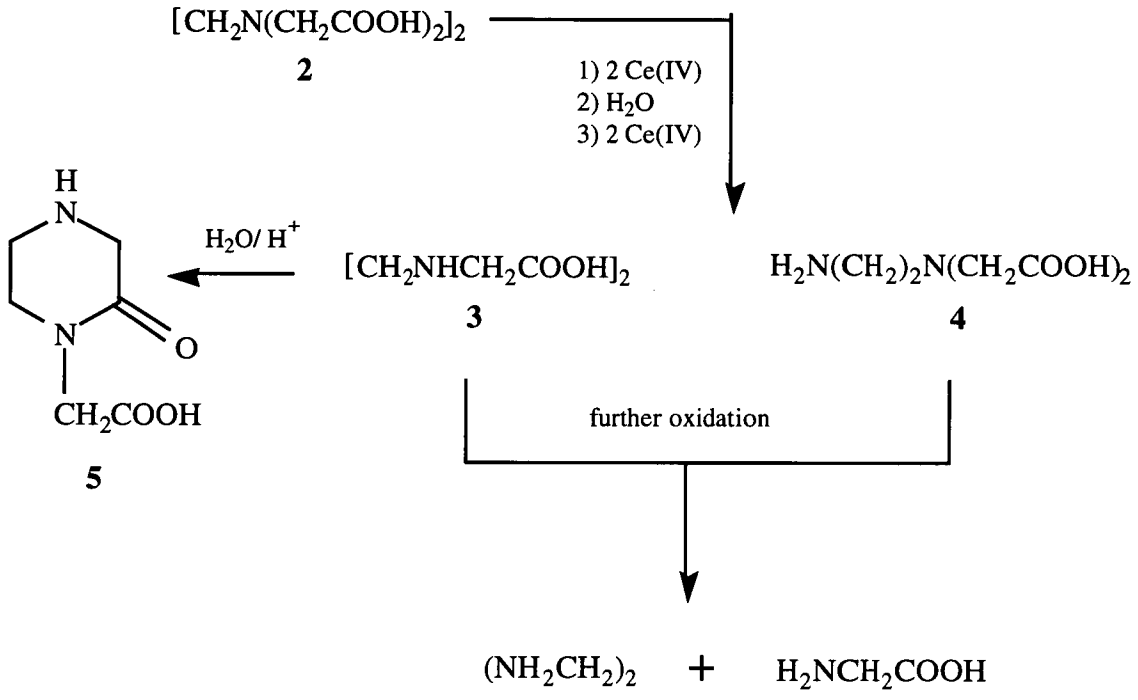


*Figure 2.2 Oxidative decarboxylation of EDTA by acidic Ce(IV)*

## 2.4 Photodegradation of DTPA

Photolysis studies involving DTPA are limited.[7,8] In comparison, photolysis of EDTA has been investigated quite extensively, due to its more widespread industrial use than DTPA.[7-11] However, whether the ligand is EDTA or DTPA, the by-products of photolysis show appreciable similarity. What is important or a prerequisite for photolysis is the *speciation* of the ligand.

A number of studies report the instability of Fe(III)-DTPA solutions in daylight; the same solutions were stable in the dark.[3,7] In one study,[3] when solutions containing ferric ions and DTPA (2:1) were illuminated a reduction in DTPA concentration from 50 mg $L^{-1}$ to nil in 14 days was observed. Decomposition of DTPA complexes commenced immediately upon illumination. A correlation between absence of iron and high levels of DTPA showed the dependence of speciation for photolysis of DTPA.

Photodecomposition of DTPA in pulping streams is likely to occur in the wastewater treatment plant, where Fe(III), light and dissolved oxygen are freely available. A schematic of the ANM (Albury) wastewater treatment plant, showing routine sampling points ($SP_x$) and pH regime, is given in Figure 2.3. Typical residence time for any given volume of water in the treatment circuit is about 6 days : primary clarifier (1 day), aeration and secondary clarifier (1 day) and holding lagoon (4 days). At pH values between 5-8, one could expect DTPA to be present as Fe(III)-DTPA, according to the pH versus stability constant curves given in Figure 2.4.

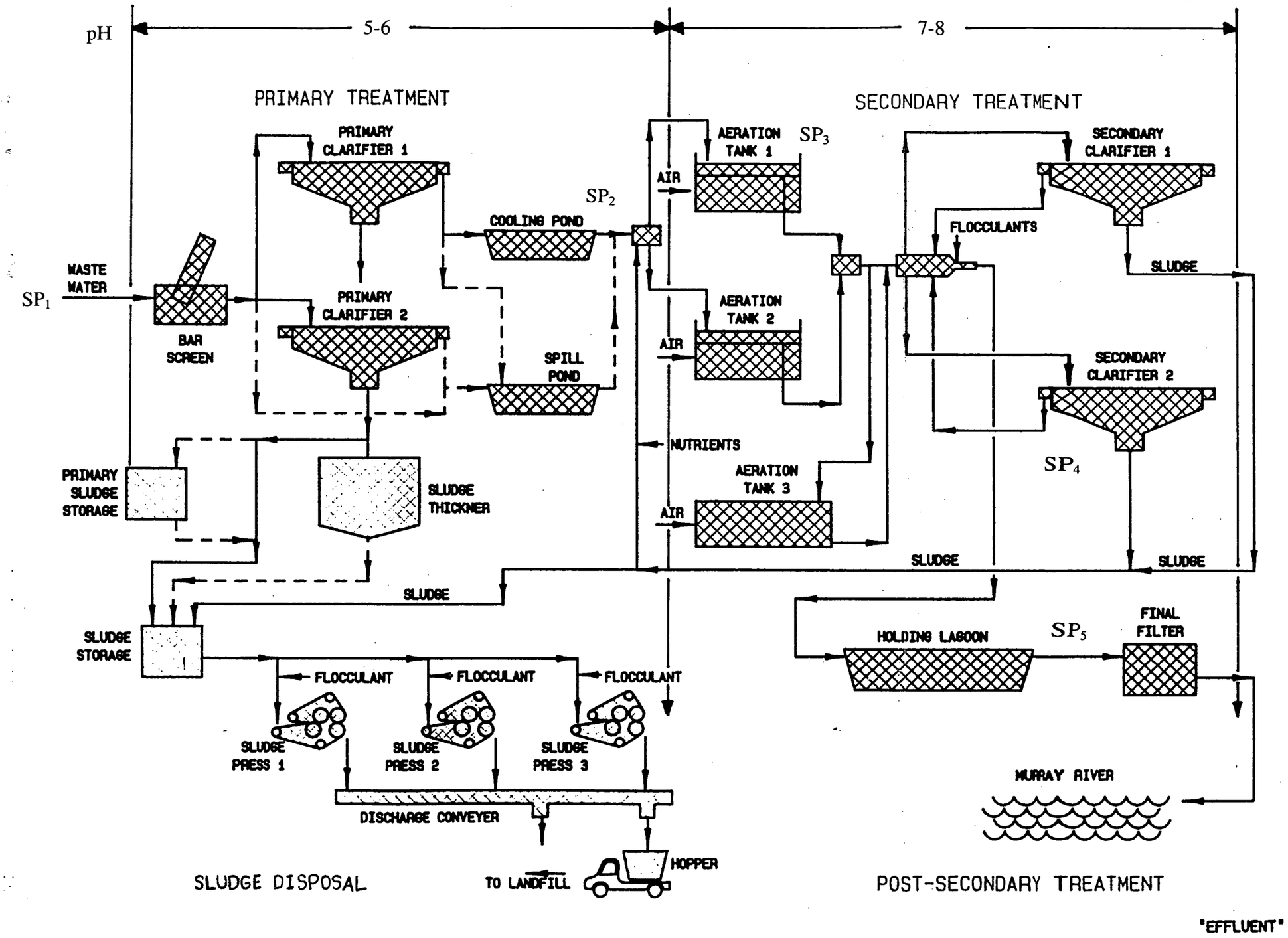


*Figure 2.3 Schematic of the ANM wastewater treatment plant*

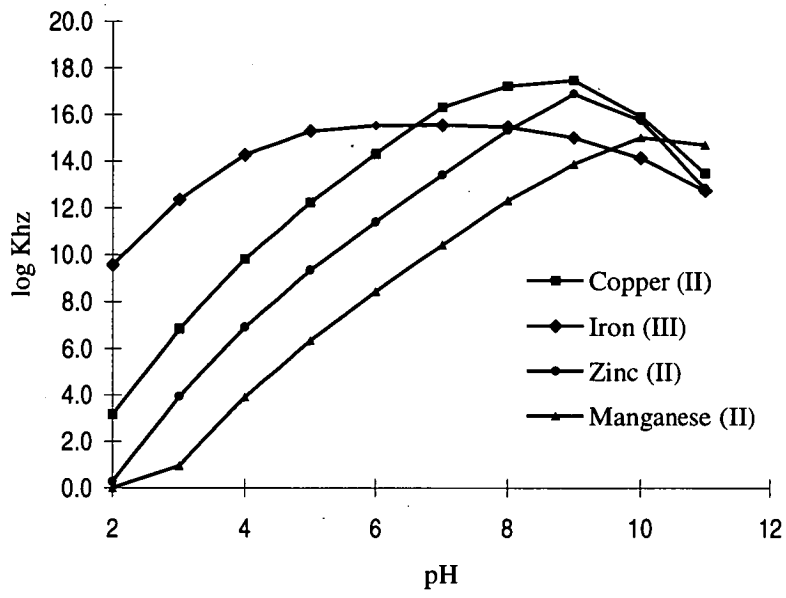


*Figure 2.4 Conditional stability constant vs pH curves for various metal-DTPA complexes*[12]

In a study[3] that monitored the fate of DTPA in pulping liquors it was found that 21% added DTPA was removed by wastewater treatment. Of this 21%, 14% reduction in DTPA was recorded across an aeration stage (biological treatment) and 7% over a holding lagoon.

Referring to Figure 2.3, the presence of suspended solids at the aeration stage would reduce penetration of the light essential for Fe(III)-DTPA photolysis and

thus limit photolytic decomposition. The decomposition occurring at this stage would most likely be of a chemical and biological nature.

There exists greater opportunity for Fe(III)-DTPA photodegradation in the holding lagoon. Removal of particulates during the preceding clarifying stages would permit greater light penetration and thus increase the likelihood of photolysis of Fe(III)-DTPA . Degradation via Mn(II)-DTPA is still likely to occur, but to a lesser extent. It should be noted that photolysis of Fe(III)-DTPA is more rapid than chemical oxidation of Mn(II)-DTPA.[3]

### *2.4.1 Photodecomposition Products of DTPA*

Several authors[10,13] have identified the major by-products of Fe(III)-EDTA photodegradation, including ethylenediaminetriacetic acid, ethylenediaminediacetic acid, formaldehyde and carbon dioxide. Other notable by-products include iminodiacetic acid (IDA), ethylene-diaminemonoacetic acid and glycine. In a recent study,[3] similar types of photolytes from Mn(II)-DTPA and Fe(III)-DTPA were identified, including cyclic species not previously observed in photolytic studies of DTPA. The stabilities of these cyclic products (eg. **1a**) were not established formally.

### *2.4.2 Determination of Photolytes*

The mono, di and tri acetic acids of ethylenediamine have been methylated and identified using gas chromatography.[10] NMR has been used to identify ethylenediaminetriacetic acid as a cobalt chloride complex.[13] Formaldehyde has been confirmed by reaction with 2,4-dinitrophenylhydrazine. Other methods for the detection and quantitation of DTPA photolytes include HPLC and MS techniques.

## 2.5 Microbial Degradation of DTPA

Biodegradation of DTPA in pulping liquors could be expected to occur in similar locations to photolysis, that is during wastewater treatment. Again, referring to Figure 2.3, microbial decomposition of pulp liquor components is certainly encouraged by the addition of nutrients and aeration during secondary treatment. However, the specific biodegradation of DTPA during wastewater treatment is expected to be poor at best. In one review[7] of NTA, EDTA and DTPA biodegradation, DTPA was found to persist during wastewater treatment. In fact, DTPA has been found to retard the decomposition of other carbon compounds, unless complexed with a metal ion.[14] In comparison, nitrilotriacetic acid (NTA) and EDTA show much more amenability to microbial breakdown. Several members of the genus *Pseudomonas* are capable of utilising NTA as the sole carbon source.[14] Until recently, bacterial strains capable of metabolising EDTA as the sole carbon source had not been identified. Strains have now been successfully isolated and enriched which use EDTA as the sole carbon and nitrogen source.[15] The microbes were sourced from industrial sewage receiving EDTA-containing wastewater.

According to another study,[16] up to 99% degradation of EDTA was achieved for a model wastewater where inflow [EDTA] was 200 mg $L^{-1}$. The output (effluent) contained in excess of 80% of the theoretically expected nitrate end product. It is important to note that the model wastewater contained a range of inorganic nutrients so EDTA was most likely coordinated with a metal. Use of mixed rather than pure cultures for removal of DTPA from wastewaters appears to be the recommendation of most studies. Some authors[15,17] contend that microbial breakdown of DTPA, EDTA and to a lesser extent NTA is poor due to the inherent chelating action which may bind certain metal cofactors ($Mn^{2+}$, $Ca^{2+}$) required by enzymes for degradative function. Furthermore, the conformation of the "free" ligand or complex may play a role in poor biodegradability.

Most of the intermediates identified when EDTA is photodegraded are also observed when EDTA is biodegraded. By inference similar intermediates are expected from microbial degradation of DTPA.

## 2.6 Thermal Degradation of DTPA

DTPA, whether free or complexed, can encounter temperatures ranging from 60°C (paper machine) to 120°C (secondary refiner) in pulping/ paper making. Published thermal degradation studies of aminopolycarboxylic acids involve predominantly EDTA[18,19] with some studies on NTA.[18] Similar information on DTPA is limited. The assumption is, however, that the thermal behaviour of DTPA will be similar to EDTA, based on the analogous behaviour of NTA and IDA.

The thermal stability of EDTA and DTPA at temperatures between 0 -100°C is well documented.[18,19] Solid phase DTPA has been shown to be stable to 120°C by static heating and thermogravimetry.[18] The same study showed sodium complexones of solid phase DTPA had increased thermal stability with increasing replacement of H by Na. A similar trend was noted in a study[20] of thermal degradation of EDTA in alkaline solutions. EDTA was considerably stabilised toward thermal decomposition through co-ordination with metal ions. However, silicate was found to catalyse the degradation of certain complexones, such as Ca(II) and Mg(II) EDTA, presumably due to the formation of insoluble Ca and Mg silicates, freeing EDTA and enhancing reaction rate.

From the preceding discussion, one would expect thermal degradation of DTPA in pulping liquors to be negligible. Although during refiner bleaching the temperature may reach 120°C at pH 10, thermal decomposition would not be favoured by the short retention time. Furthermore, as indicated previously the thermal stability of coordinated DTPA can exceed that of the free ligand.

## 2.7 Summary

Various processes by which DTPA may be degraded in pulping liquors and wastewaters have been presented. Reaction products for each particular breakdown process have been described, as have techniques for their analysis and detection. The similarity between breakdown products of both EDTA and DTPA caused by chemical oxidation, photolytic and biological degradation suggest strongly that a universal mechanism is responsible for their decomposition. From the preceding discussion there is strong evidence to suggest that **1a** is a product of *successive oxidative decarboxylation* of DTPA. The preceding investigation of how DTPA might be degraded in pulping liquors will provide valuable leads toward developing methods for the synthesis of **1a**.

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# CHAPTER 3

## Proposed Methods for Preparation of Cyclic DTPA Degradation Products

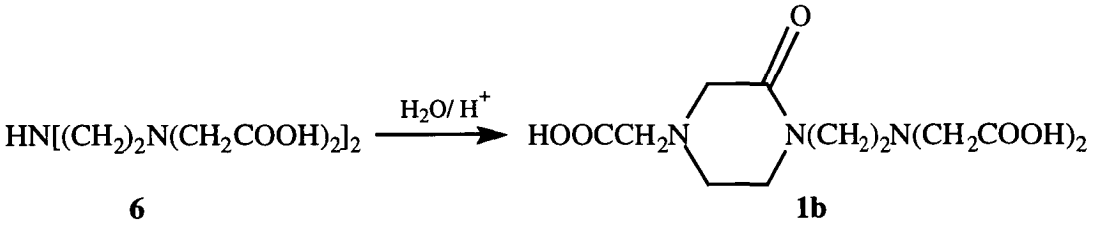
### 3.1 Introduction

#### *3.1.1 Current Preparation of Cyclic DTPA Degradation Products*

It is well known that chelating agents of the aminopolycarboxylic acid type are rapidly photolytically oxidised in the presence of the ferric ion.[1] ANM researchers confirmed this relationship[2] using DTPA then extended the study to include the effects of other metal ions, namely $Zn^{2+}$, $Mn^{2+}$ and $Bi^{3+}$. The key finding of their work was that $Mn^{2+}$ promoted the decomposition of DTPA independent of light. This observation led to the preparation of DTPA degradation products (including **1a**) by direct permanganate oxidation of DTPA in acidic solution. Unfortunately this preparative approach has limited practical application due to the formation of a complex mixture of compounds. Thus more elegant and reliable organic synthetic methods must be developed for the preparation of **1a**.

The structural isomer of **1a**, 1-[2-[bis(carboxymethyl)amino]ethyl]-4-(carboxymethyl)-2-oxopiperazine (**1b**), has been prepared by at least two independent methods.[3,4] In one study,[4] **1b** was produced by debenzylation of *N'*-benzyldiethylenetriaminetetraacetic acid in the presence of palladium and hydrogen at room temperature. A yield for the conversion was not reported. Other experimental details not reported included analytical quantities used, work-up procedures and the percent loading of palladium on the Pd-C catalyst. In another study,[3] researchers at Akzo Nobel (manufacturer of DTPA) prepared **6** initially

then achieved cyclisation in acidic media to **1b** (Figure 3.1). Further details of this method have not been published to date. In comparison, though at least two groups[2,5] have detected and identified the presence of DTPA degradation products (including **1a** and **1b**) in wastewaters, methods for complete synthesis of **1a** have not been found. A number of methods for the preparation of **1a** by organic synthesis are now presented for discussion.




**6** **1b**

*Figure 3.1 Partial scheme for preparation of* ***1b***

## 3.2 Unit Structures of the Cyclic DTPA Degradation Product

### *3.2.1 Piperazinone : A Possible Starting Point*

An outstanding feature of **1a** is the cyclic unit, the construction of which is viewed as the logical starting point and perhaps the most difficult part of the synthesis. Substituents can then be attached to this ring structure over subsequent steps. In its most basic form the cyclic unit is piperazinone (**7**, Figure 3.2). This compound is not available commercially but may be synthesised readily from relatively common reagents, as depicted in Figure 3.2. Thus treatment of ethyl chloroacetate with a large excess of ethylenediamine leads to the formation of a monoalkylethylenediamine, which is then cyclised to **7** under high temperature and vacuum. Typical yields reported for **3** range between 39-50%.[6,7]

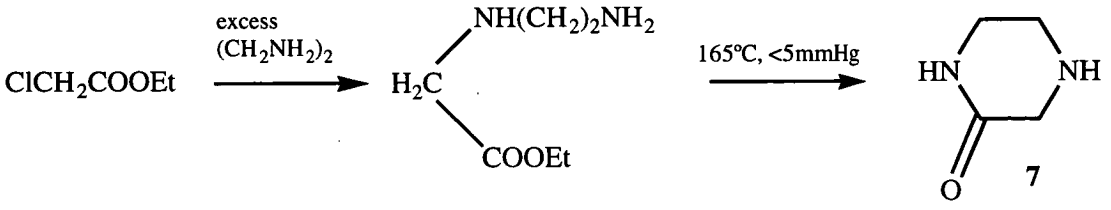
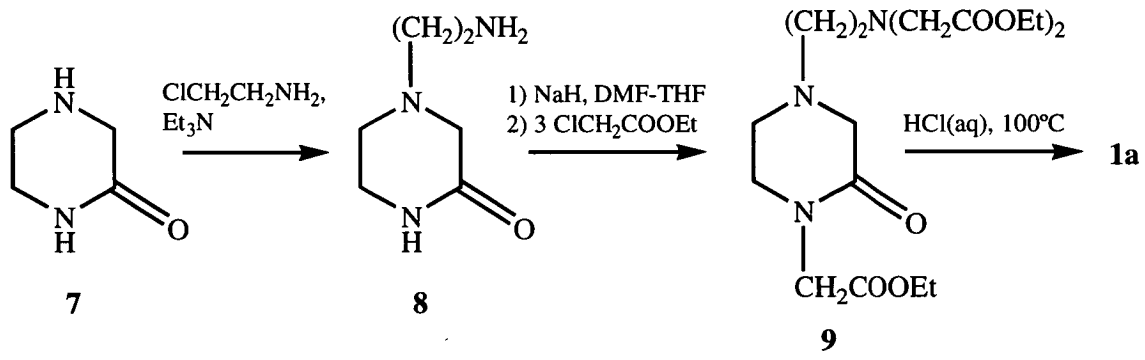



*Figure 3.2 Preparation of piperazinone*

Due to keen and sustained interest in piperazinone derivatives as pharmacologically active agents, many synthetic methods involving **7** are available,[8-10] several of which have particular relevance to the current application. In most cases **7** is not actually prepared as a discrete compound. Rather, the condensation reactions usually employed to produce piperazinone compounds give rise to substituents at N-1 and N-4. Such condensation reactions are perhaps more efficient than forming **7** then alkylating. For this to be realised, however, great care must be taken when selecting linear precursors.

A possible route for the preparation of **1a** using **7** as precursor is outlined in Scheme I. After preparation of **7**, the aim will be to alkylate N-1 using 2-chloroethylamine HCl to give **8**. Following activation of N-4 with sodium hydride, **8** may be treated with three equivalents of ethyl chloroacetate, giving the ethyl ester of **1a**. Mild acid hydrolysis of **9** would then lead to the desired cyclic DTPA degradation product.




*Scheme I*

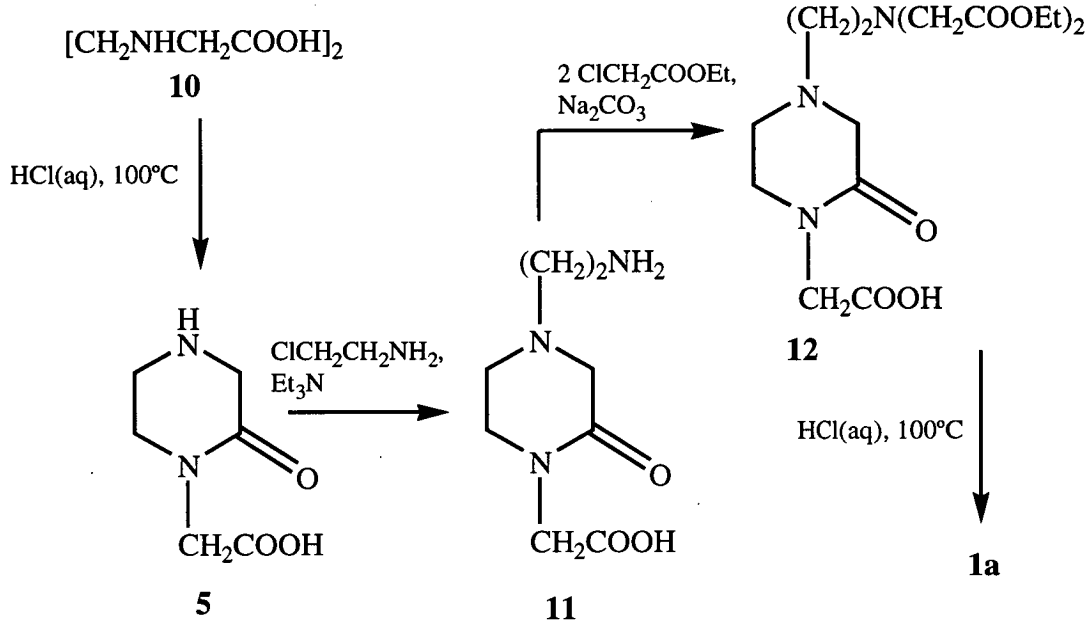
The limitations inherent in Scheme I should be noted. First, the step involving 2-chloroethylamine has not been verified previously and thus time must be devoted to its development. Second, access to specialist apparatus, such as a high vacuum single path distillation unit (eg. Kugelröhr apparatus) is required for production of **7**. Other limitations include the likely poor efficiency of alkylation at N-4 and the requirement to operate under strictly anhydrous conditions during this step. Despite the identified limitations, **7** would serve as a very useful precursor or intermediate for **1a** and thus Scheme I will be explored.

### *3.2.2 2-Oxo-1-piperazineacetic acid : A Better Starting Point ?*

The other simple cyclic unit contained in **1a** is 2-oxo-1-piperazineacetic acid (S-KP, **5**), which was previously noted (Figure 2.2) as an oxidation product of EDTA [it is worth noting that **1b** contains 3-oxo-1-piperazineacetic acid (U-KP), which may also be derived from EDTA]. In practice, **5** is prepared by treatment of ethylenediamine-*N*,*N*′-diacetic acid (**10**) with hot aqueous acid.[11,12] Treatment of **5** with hot alkali regenerates **10**. Whilst several authors[11-13] have described this interconversion, interest in **5** as a potential precursor to novel aminopolycarboxylic acid ligands has been limited. Although similar in

appearance to **7**, **5** shows no pharmacological activity. The acid dissociation constant for **5** has been determined, (Chapter 5) in addition to a limited number of stability constants with transition metals.[11]

A possible route for the preparation of **1a** using **5** as an intermediate is shown in Scheme II.




*Scheme II*

In essence Scheme II is analogous to Scheme I proposed for piperazinone. However, Scheme II appears to offer several distinct advantages over Scheme I, namely :

- a reduction in the number of steps, since there is no need for separate alkylation at N-4 (cyclisation leads to an intermediate with a carboxymethyl

substituent at N-4)

- **10** is commercially available
- as fewer steps are required, time and resources will be better utilised

The disadvantages relating to the untested step involving 2-chloroethylamine still clearly apply to Scheme II. Another possible limitation of this scheme relates to the high cost of **10**, which may be prohibitive considering the quantity of **1a** required for subsequent complexing studies. The high cost of **10** could be offset by preparing quantities of this amino acid in-house using the method outlined in Figure 3.3. Thus ethylenediamine is alkylated with bromomalonic acid (**14**)[14] to give *N,N'*-ethylenebis(aminomalonic) acid (**15**) which is then decarboxylated to **10**.[15] Since decarboxylation is performed in acidic conditions, some **10** is cyclised to give **5** as a minor product. The overall yield of **10** from **13** was not reported, but a yield of 60% has been quoted for **14**.[14]

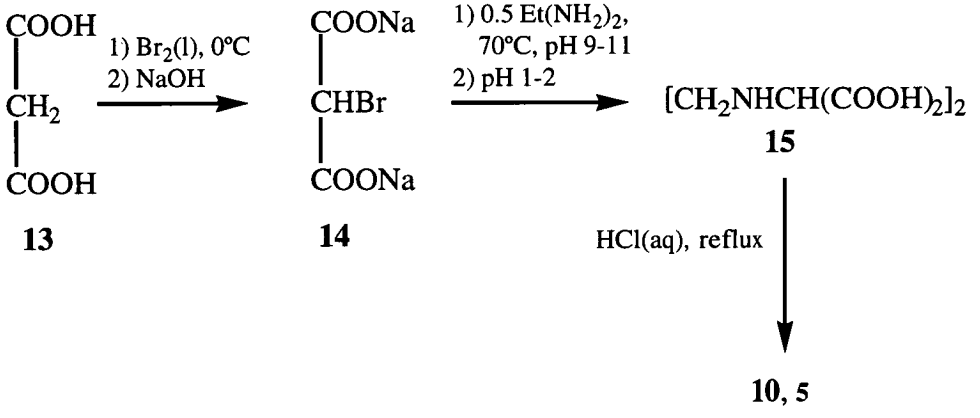



*Figure 3.3 In-house preparation of **10** via **15***

### 3.3 Other Approaches for Preparation of Alkyl Substituents

Both Schemes I and II propose to *N*-alkylate compounds **5** and **7** in a series of steps. Another approach that could prove more efficient involves preparing the entire alkyl substituent *prior to* reaction with N-1. An appropriate alkyl substituent would be dimethyl 2-chloroethyliminodiacetate (**17**), prepared from the corresponding dinitrile with gaseous HCl in methanol.[16] Preparation of 2-chloroethyliminodiacetonitrile (**16**) is by Mannich's reaction and a yield of 60% has been reported.[16] The synthesis of **17** is shown in Figure 3.4. Figure 3.5 then illustrates how either piperazinone or 2-oxo-1-piperazineacetic acid may be alkylated with **17**. In the case of alkylating **5** with **17**, mild acid hydrolysis would be expected to give **1a**.

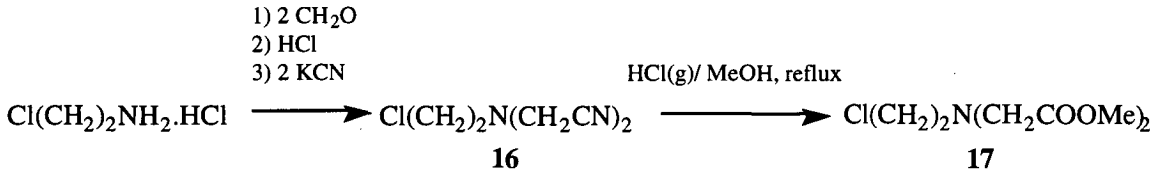



*Figure 3.4 Synthesis of alkyl substituent* ***17***

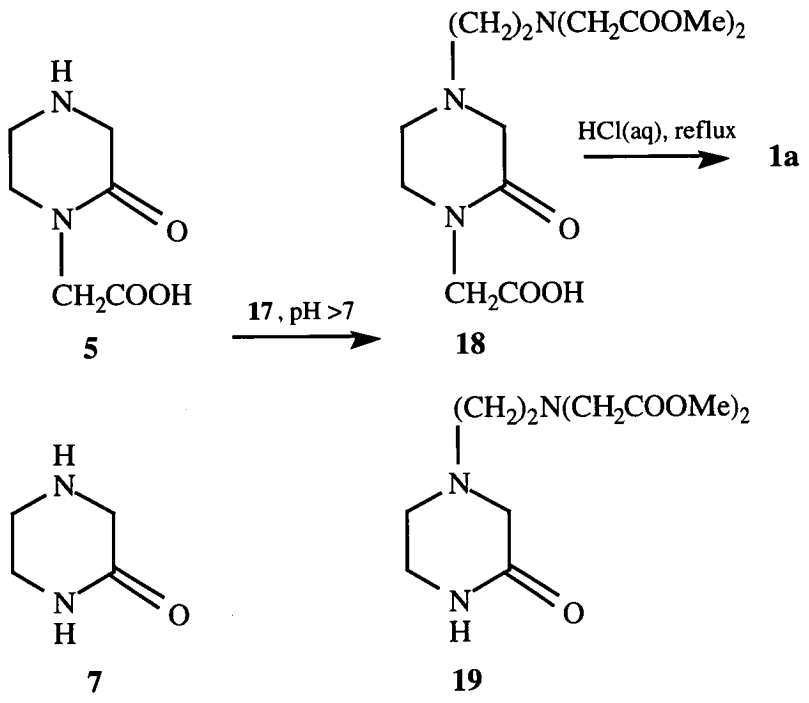



*Figure 3.5* N-*alkylation with* **17**

Another, though less attractive way, of producing the entire alkyl substituent could be to use hydroxyethyliminodiacetic acid (HEIDA, **20**). HEIDA itself will not alkylate either **5** or **7** unless its reactivity is enhanced. Activation could be achieved by exchanging the hydroxy group for a more suitable leaving group (such as tosyl or triflate) and converting the carboxy groups to a more neutral (eg. ester) species. This modification would essentially produce a compound similar to **17**. However, the conversion of **20** is not expected to occur readily. Figure 3.6 outlines two possible means of making **20** suitable for alkylation with either **5** or **7**. These conversions have not been documented previously.

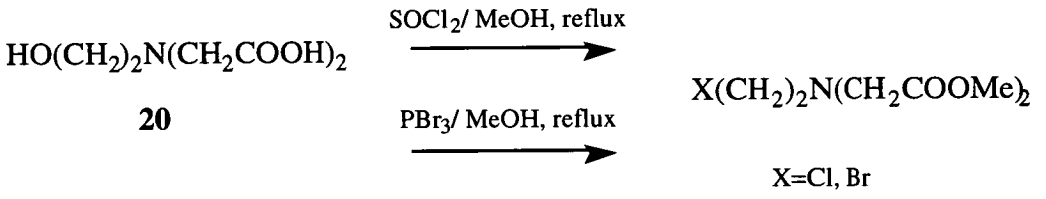



*Figure 3.6 Alkylating group from* ***20***

Several distinct advantages can be identified by preparing the entire alkyl substituent separately, rather than building onto **5** or **7** in steps, including :

- methods for compounds **5**, **7** and **17** are known
- the integrity of the cyclic units is maintained by not performing the alkylation until the last possible moment
- work-up procedures are likely to be more straightforward

Thus, separate production of the entire alkyl substituent removes some of the uncertainty associated with the synthesis and allows for greater flexibility within the method. Whilst separate production of the alkyl sidechain may ultimately be more efficient, the practical merits of both approaches will be explored fully.

## 3.4 Other Synthetic Methods

### *3.4.1 Diethylenetriamine-*N,N′,N″*-triacetic acid*

It is clear from Figure 3.7 that diethylenetriamine-*N,N′,N″*-triacetic acid (**21**) shows considerable structural similarity to **10**. It follows then that **21** may behave in an analogous manner to **10** when treated with aqueous acid, leading to the cyclic species indicated. Indeed, as related in section 3.1.1, **6** has been cyclised in acidic conditions to produce the oxopiperazinetricarboxylic acid **1b**. Another

study,[5] where both **1a** and **1b** were detected in river waters receiving industrial effluent, postulated the formation of **1b** (and **1a**) via a similar mechanism.

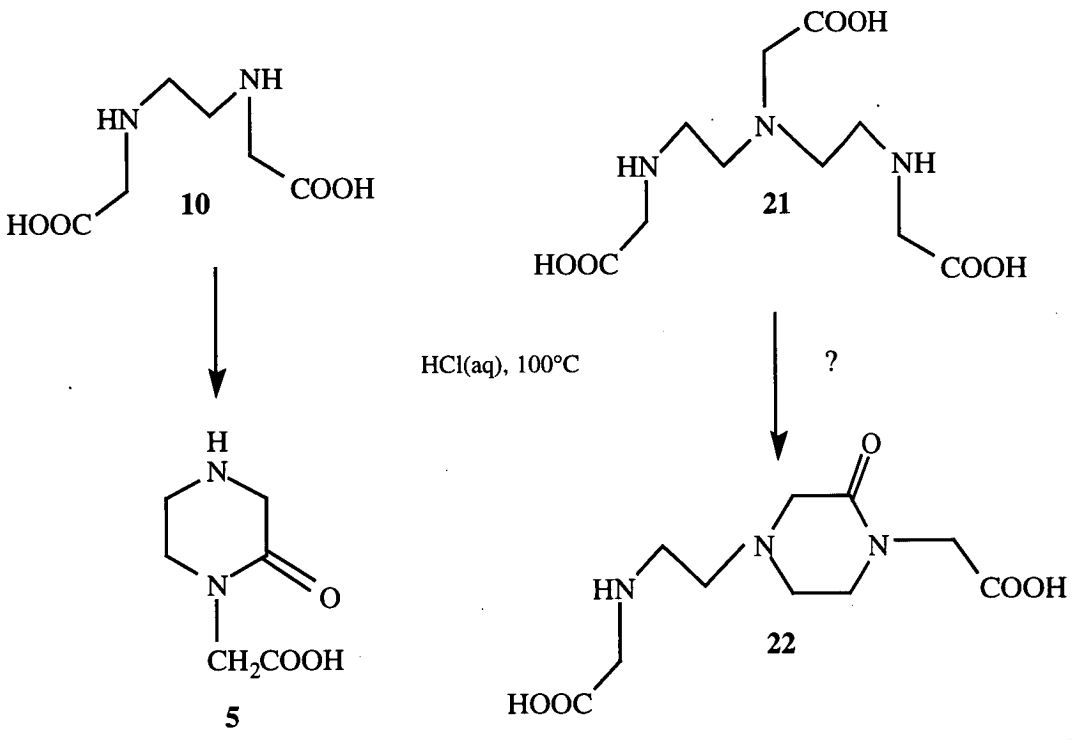
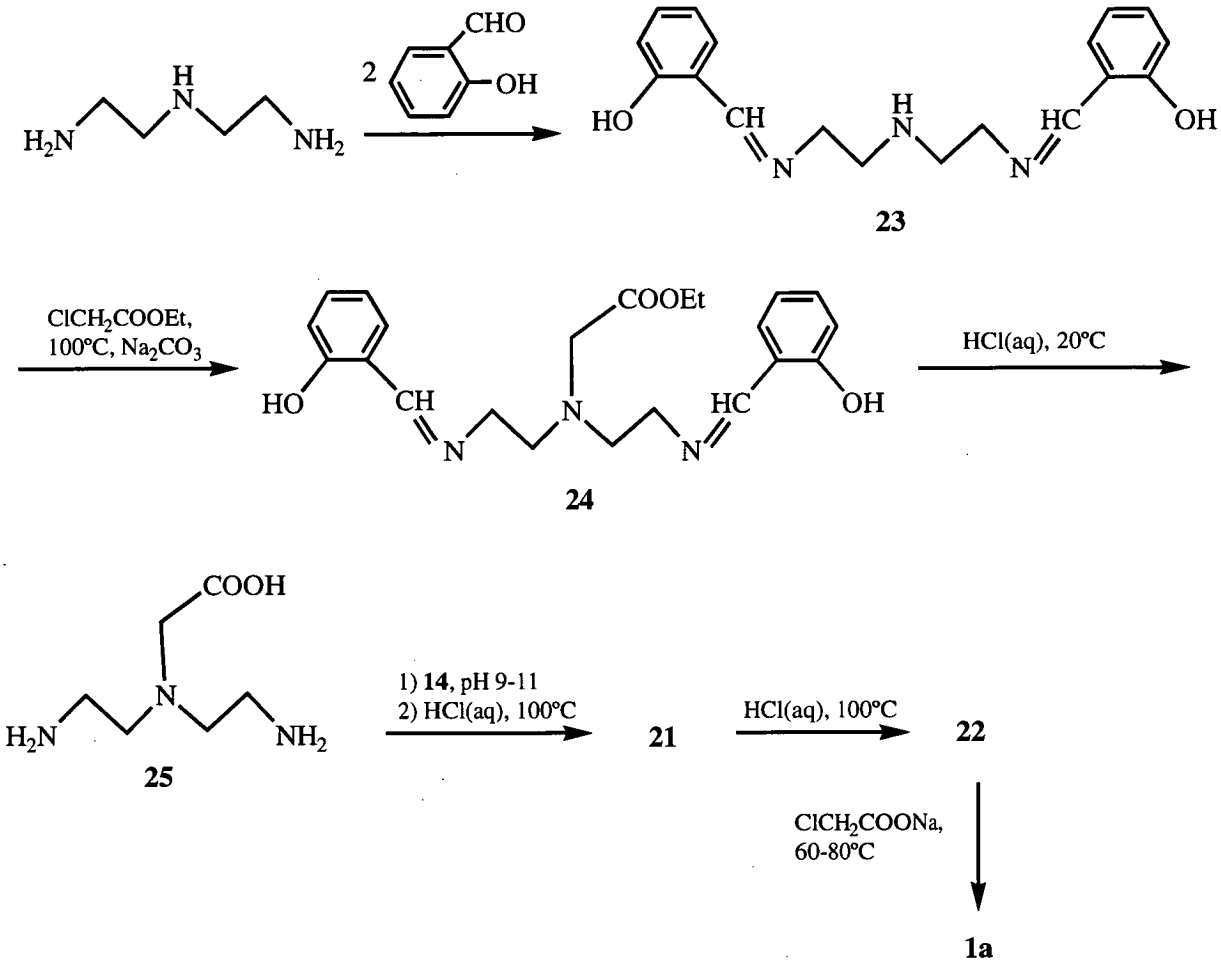



*Figure 3.7 Proposed cyclisation of **21** with hot acid*

A possible method for the synthesis of **1a** via **21** is illustrated in Scheme III. The Schiff's base (**23**) is firstly synthesised[17] then alkylated with ethyl chloroacetate to give **24**. Mild aqueous acid hydrolysis of **24** leads to diethylenetriamine-*N'*-acetic acid (**25**). Diethylenetriamine-*N,N',N''*-triacetic acid (**21**) is produced by treating **25** with two equivalents of **14** then decarboxylating.[18] Further exposure of **21** to hot acid is postulated to give **22**. The desired cyclic DTPA degradation product is then obtained by reacting **22** with excess sodium chloroacetate. Alternatively, **22** could be treated with ethyl chloroacetate then hydrolysed to give **1a**.




*Scheme III*

Of the three general schemes proposed for the synthesis of **1a**, Scheme III appears the least favourable because :

- at least two steps have not been tested by experiment, the most significant being conversion of **21** to **22**
- the reaction route involves more steps than either Scheme I or II, thus reducing

efficiency

- previous studies[18,19] suggest that mixtures of amino acid intermediates can be very difficult to separate and thus purify

Although all schemes will be explored, the main focus will be directed toward I and II, where the syntheses are shorter, better documented and thus more likely to succeed.

## 3.5 Summary

At least three different approaches have been proposed for the synthesis of cyclic DTPA degradation product **1a**. The merits of each approach have been discussed and will be evaluated by experiment. It is quite possible that more than one method will be identified for the production of **1a**. However, only the most practical will be developed fully. Whatever method is devised, isolation of amino acid intermediates is expected to be challenging, due to their amphoteric nature. Previously, crystallisation techniques have been used with success for purification of compounds including **5**, **10**[12] and **15**.[15] However, both **21** and **25** have not been isolated as free acids by crystallisation.[18,19] In cases where crystallisation is not effective the envisaged purification techniques will include chromatography, distillation and solvent extraction.

## 3.6 References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# CHAPTER 4

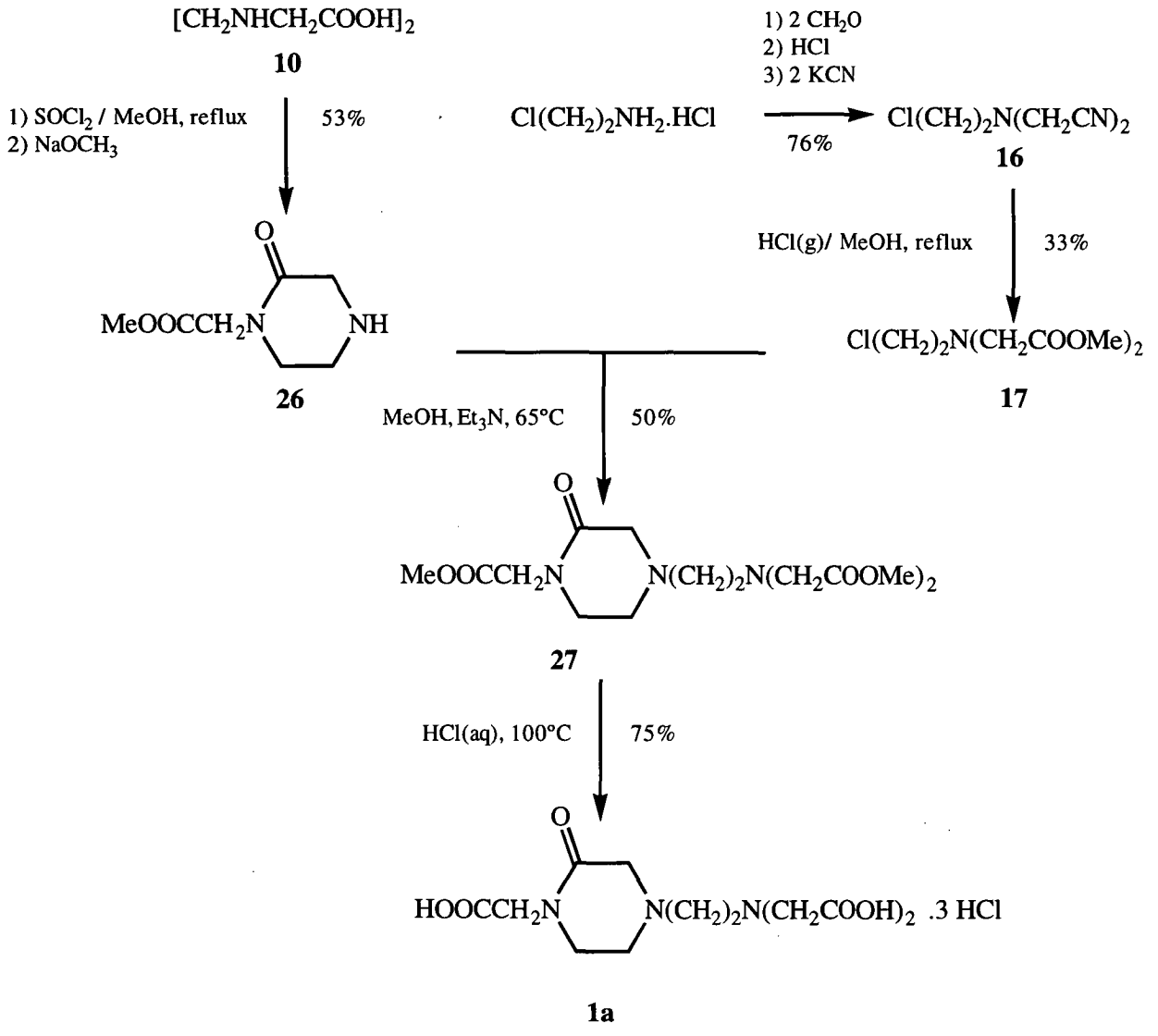
## Synthesis of the Cyclic DTPA Degradation Product

### 4.1 Introduction

In Chapter 3 several methods were proposed for the preparation of **1a**. These methods were evaluated by experiment and the results are now presented.

Practical evaluation found the most appropriate method for synthesis of **1a** to be a modification of Scheme II (Scheme II′). As this scheme was ultimately selected for the production of **1a,** its development will be discussed first. The alternative proposals, which were explored in some depth and did assist in the development of Scheme II′, will then be presented. These alternative pathways were explored as part of a general effort to produce **1a** and led ultimately to the selection of Scheme II′. Whilst it may be entirely possible to prepare **1a** by the alternative approaches which will be presented, these methods suffered from a number of crucial disadvantages. Thus it will be shown in the following section how the synthetic approach evolved toward Scheme II′. It should also be re emphasised that ultimately only one viable method was required, as it was not the synthetic pathway of **1a** which was the focus but the determination of stability constants.

Detailed experimental methods (including analytical data) for each compound are given in Chapter 7.


[CH2NHCH2COOH]2
10
1) SOCl2 / MeOH, reflux
2) NaOCH3
53%
MeOOCCH2N
NH
26
Cl(CH2)2NH2.HCl
1) 2 CH2O
2) HCl
3) 2 KCN
76%
Cl(CH2)2N(CH2CN)2
16
HCl(g)/ MeOH, reflux
33%
Cl(CH2)2N(CH2COOMe)2
17
MeOH, Et3N, 65°C
50%
MeOOCCH2N
N(CH2)2N(CH2COOMe)2
27
HCl(aq), 100°C
75%
HOOCCH2N
N(CH2)2N(CH2COOH)2 .3 HCl
1a

*Scheme II'*

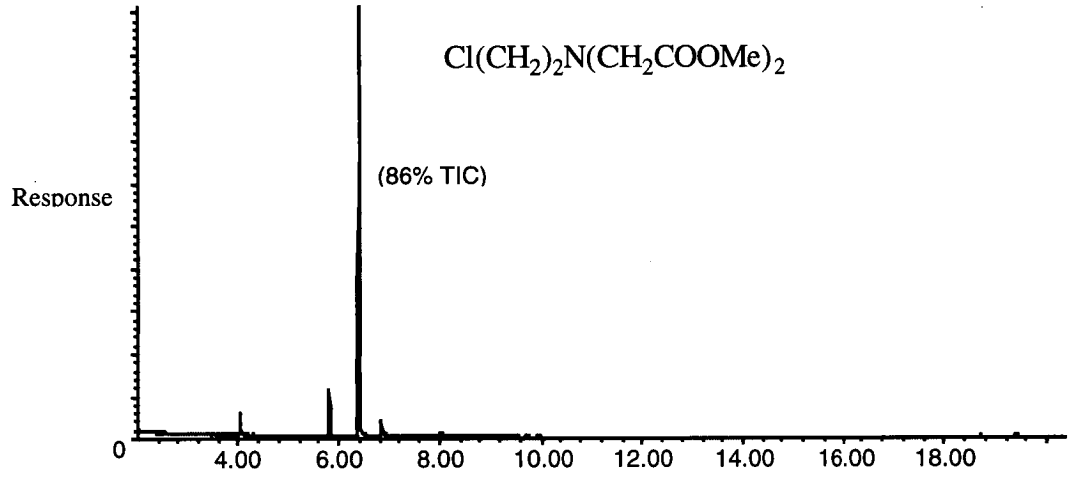
## 4.2 Scheme II´

Published methods were followed, with some modifications, to produce compounds **5**,[1] **14**,[2] **15**,[3] **16**, **17**[4] and **26**.[1] Note that compounds **5**, **14** and **15** are not shown in Scheme II′; their synthesis was detailed previously (Figure 3.3). No deliberate attempts were made to optimise yields.

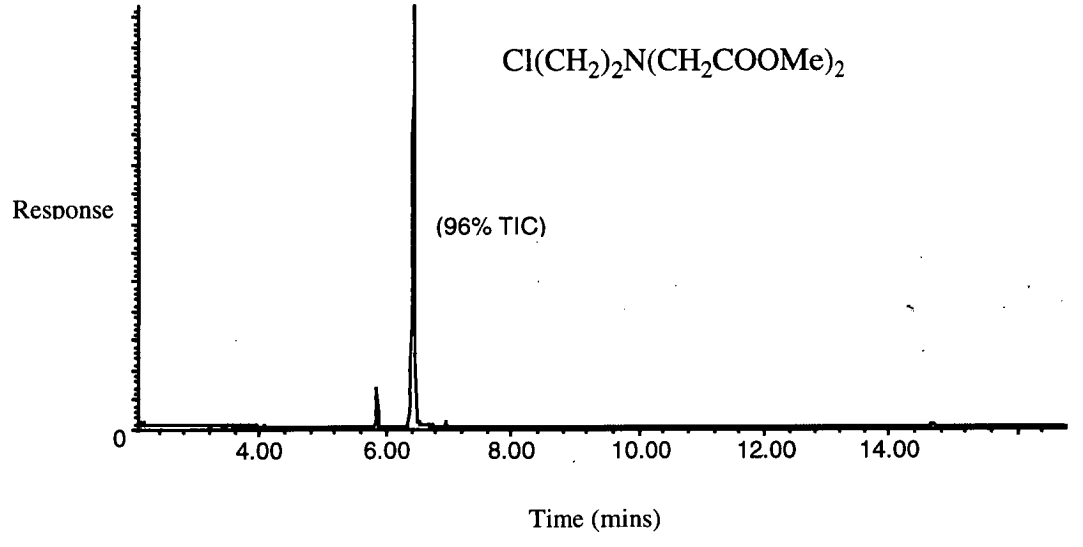
Ethylenediamine-*N*,*N*′-diacetic acid (**10**), obtained either commercially or from decarboxylation of *N*,*N*′-ethylenebis(aminomalonic) acid (**15**) (refer Figure 3.3), was cyclised to 1-methoxycarbonylmethyl-2-oxopiperazine hydrochloride in hot $SOCl_2$/MeOH. The free base (**26**) was liberated by treatment with the calculated amount of $NaOCH_3$. The yield of **26** increased with increased reaction time, for example, given the same quantity of **10** (10.0g) the yield of the monohydrochloride of **26** was 39.0% after 30 h and 59.6% after 36 h.

Dinitrile **16** could be manufactured rapidly "in bulk" in yields up to 76%, a gain of about 16% on a previous publication.[4] The improvement in yield could be attributed to both a slower rate of addition of reactants to 2-chloroethylamine HCl and more efficient means of mixing (paddle stirrer). However, such yields could not be achieved with the following conversion of **16** to **17**. In a previous report[4] 100g of **16** was converted to **17** in 9 h. In the present case, a similar period was required for conversion of only 10g. If shorter reaction times were employed then the reaction solution contained substantial amounts of the mono ester. It was also determined that improved yields of **17** could be obtained by a work-up on silica gel, rather than by high temperature/ high vacuum distillation, which resulted in considerable decomposition. It should also be noted that neither high temperature/high vacuum distillation nor the given chromatographic work-up was able to furnish pure **17** if the mono ester was present to any great extent. Nevertheless, the mono ester could quite readily be converted to **17** by further treatment with HCl(g)/MeOH. Yields of **17** ranged between 30-33%. Figure 4.1

shows typical GCMS chromatograms of **17** as percent of Total Ion Current (TIC) before and after work-up on silica gel.




**a)**




**b)**

*Figure 4.1 GC of **17** before (**a**) and after (**b**) work-up on silica. See section 7.4 for GC conditions*

*N*-alkylation of **26** with **17** occurred readily in dry methanol at gentle reflux, as monitored by TLC and GCMS. Liberated HCl was effectively buffered by $Et_3N$ and $Et_3N.HCl$ was removed during the work-up using dry $(CH_3)_2CO$ ($Et_3N.HCl$ remains insoluble). No other reaction media were investigated, though dichloromethane and toluene could serve as suitable alternatives to methanol. After removing residual amounts of starting esters by an $(CH_3CH_2)_2O$ extraction, crude **27** usually required treatment with activated carbon before purification on silica gel (eluent $CHCl_3$:EtOH 99:1). The ester **27** was obtained as a light red oil in about 50% yield. Figure 4.2 shows a GCMS chromatogram of **27** after work-up on silica gel.

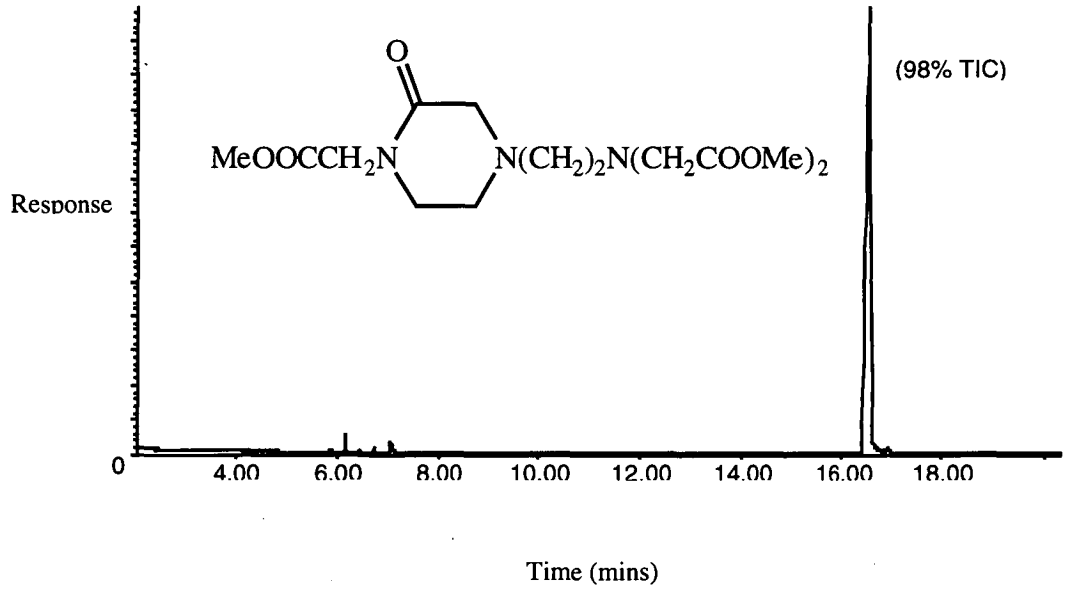



*Figure 4.2 GC of* ***27*** *after work-up on silica gel. GC conditions as per Figure 4.1.*

Mild aqueous acid hydrolysis of **27** furnished **1a** as the trihydrochloride salt in 75.2% yield. It should be noted that several peaks in the $^1H$ and $^{13}C$ NMR spectra

of **1a** could not be assigned to **1a**, indicating the presence of a small percentage of impurity (refer Chapter 6). However, no further evidence of an impurity in **1a** could be discerned from either LSIMS or elemental analyses.

Acceptable micro analyses, $^{1}H$ and $^{13}C$ NMR and MS data were obtained for most compounds and are given in the experimental section (Chapter 7).

### 4.3 Determination of 1a in the Pulp Mill Environment

As a means of relating the organic synthesis of **1a** to its actual determination in pulping wastewaters, an aqueous sample of **1a** was analysed using HPLC.[5] The method relied on the formation of the ferric complex of **1a** which was then detected at 258nm. A peak corresponding to Fe(III)-**1a** was eluted at the expected retention time and UV spectra recorded across the peak gave matching overlays, thereby showing peak homogeneity. A typical chromatogram showing elution of Fe(III)-**1a** is illustrated in Figure 4.3. All other peaks were system artifacts, as determined by blank injections.

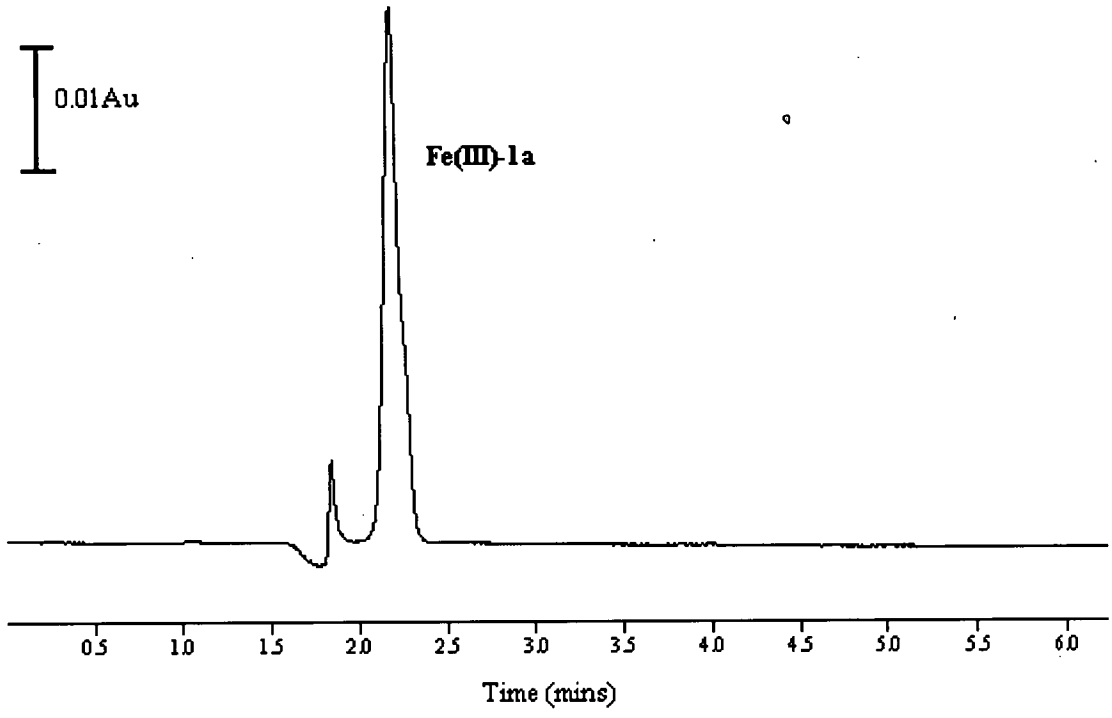


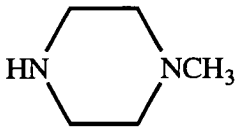

*Figure 4.3 Liquid chromatogram of* ***1a*** *as its ferric complex. See section 7.4 for HPLC conditions.*

## 4.4 Alternative Schemes

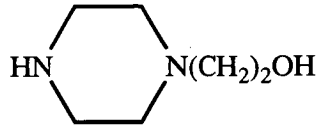
## 4.5 Scheme I

Synthesis of **7** was performed by adapting several known methods.[6-8] Although yields up to 50% have been reported,[7] the author's best was 36%. Single path distillation of the monoalkylethylenediamine using a Kugelröhr apparatus furnished pure **7** without the requirement for recrystallisation with ethyl acetate. As indicated in Scheme I (section 3.2.1), N-4 alkylation of **7** with 2-chloroethylamine had not been documented previously. To conserve **7**, the likelihood of N-4 alkylation was initially evaluated using the commercial model

compounds *N*-methylpiperazine (**28**) and *N*-(2-hydroxyethyl)piperazine (**29**) shown in Figure 4.4. Unfortunately, model compounds with an alpha keto group could not be sourced. However, both compounds satisfied the minimum criterion of containing a free secondary amino group available for N-4 alkylation.




**28**




**29**

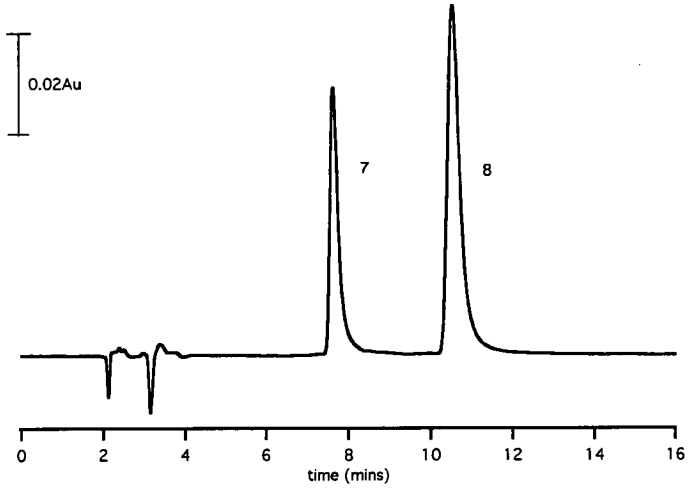
*Figure 4.4 Model compounds for N-4 alkylation test*

Model N-4 alkylations were performed typically in either dry ethanol or aqueous media at 60-80°C with pH control. Excess 2-chloroethylamine was used but gave no better results than equivalent mole ratios of reactants. It was quickly established by CIMS (refer Chapter 7) and $^1$H NMR that both **28** and **29** could be alkylated at N-4 with 2-chloroethylamine, though to what extent was not established (no attempts were made to isolate alkylated products, since this was not intended). Since the work with model compounds suggested **7** may indeed be alkylated at N-4 with the given agent, all subsequent work was performed with **7**.

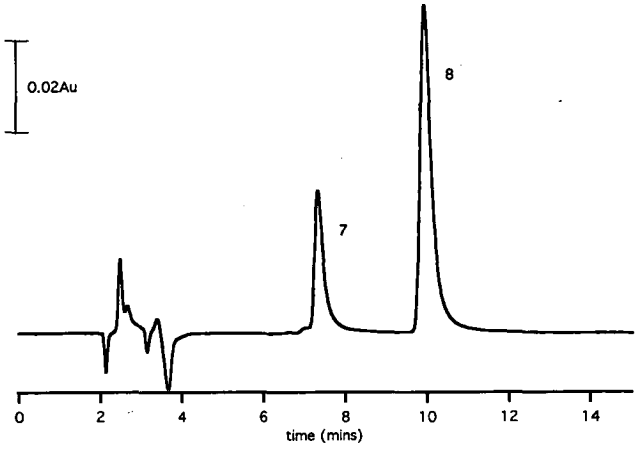
### *4.5.1 Chromatography*

As a means of monitoring (and perhaps isolating) N-4 alkylation of **7** and possibly subsequent reactions, a liquid chromatographic method was developed. Piperazinone (**7**) and related compounds were detected using a Waters 486 detector operating at 214nm. The analytical column (250 x 4.6mm) was an Activon Goldpak 5µm $C_{18}$. The retention behaviour of **7** was manipulated solely through compositional changes to the mobile phase. Under the given conditions (refer section 7.4.2) the retention factor for **7** was about 3.8, which was acceptable

for the monitoring application. N-4 alkylated products (eg. **8**) showed greater retention factors. Typical chromatograms of an N-4 alkylation mixture are illustrated in Figure 4.5. The alkylation appeared to perform better in aqueous media than dry ethanol, in that a greater degree of completeness was observed. Other organic solvents were not tested. No evidence to indicate alkylation at N-1 was found.




**a)**



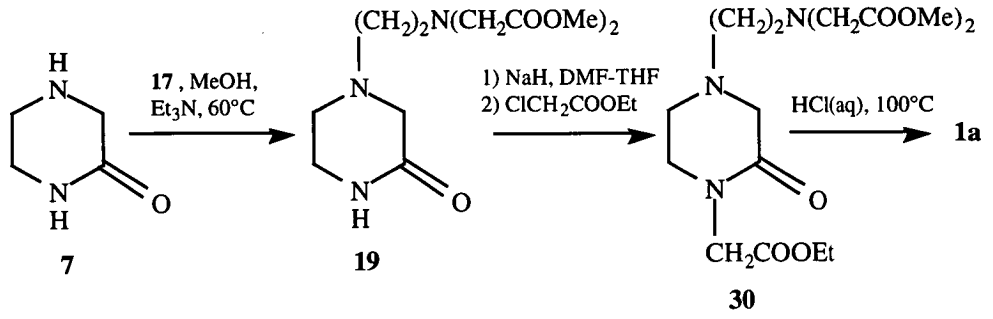

**b)**

*Figure 4.5 Alkylation of **7** in aqueous media at 4 h (**a**) and 6 h (**b**). See section 7.4 for HPLC conditions.*

By collecting the fraction suspected as the N-4 alkylated product (**8**) from the HPLC column it was possible to identify by high resolution EIMS a species with the desired empirical formula $C_6H_{13}N_3O$. Although several attempts were made to isolate this species using conventional techniques (eg. solvent extraction, crystallisation) little success was achieved. Thus, yields and other defining data were not obtained for this N-4 alkylation.

## 4.6 Scheme I′

The inability to isolate pure quantities of **8** on a macro scale caused a reappraisal of Scheme I. A decision was made to adopt the alternative approach to N-4 alkylation as outlined in section 3.3. Thus 2-chloroethylamine was replaced by **17** as the alkylating agent; this modification is shown in the revised method (Scheme I′) below. The potential benefits of this approach have been highlighted previously and several were realised in practice.




*Scheme I'*

Alkylation of **7** at N-4 with **17** proceeded smoothly under the given conditions. Triethylamine HCl was removed by treatment with dry $(CH_3)_2CO$ and residual starting materials with $(CH_3CH_2)_2O/CH_2Cl_2$ extractions. The aqueous fraction contained the majority of **19** which, after drying, was further purified by silica gel chromatography (eluent $CH_2Cl_2$:EtOH 95:5) to give **19** as a pale yellow oil in yields up to 32.0%. A typical GCMS chromatogram of **19** after work-up on silica gel is shown in Figure 4.6.

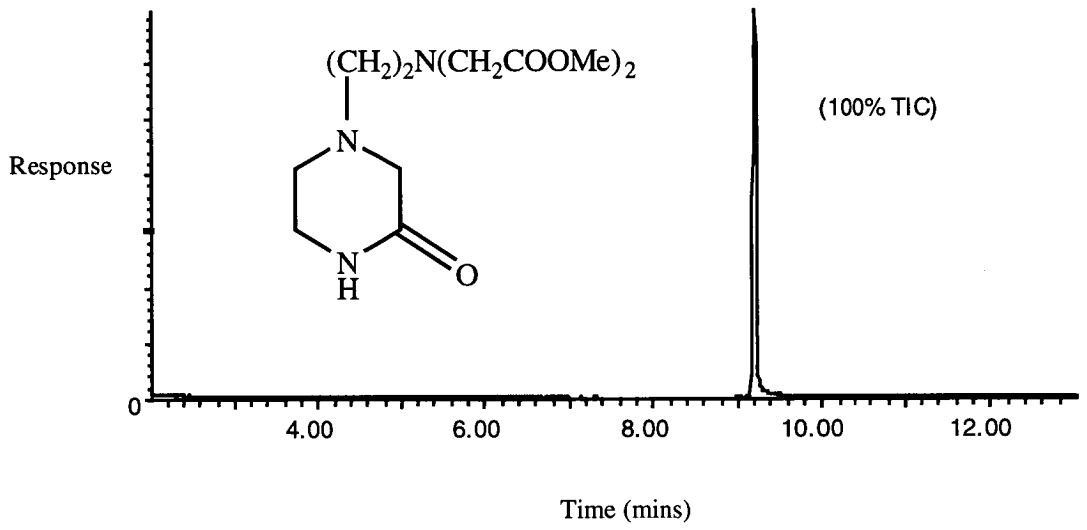
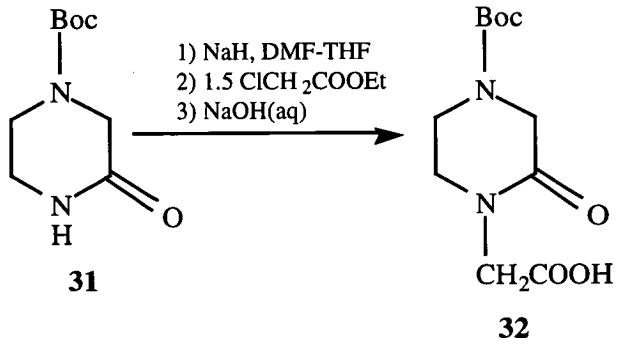



*Figure 4.6 GC of **19** after silica gel work-up. GC conditions as per Figure 4.1.*

Alkylation of **19** at N-1, in contrast to N-4, proved extremely difficult due to the deactivating effect of the ring carbonyl group and preliminary experiments were largely unsuccessful. A previous study[9] was then located (Figure 4.8) where 4-benzyloxycarbonyl-2-oxopiperazine (**31**) was treated with NaH/ $ClCH_2COOEt$ followed by mild alkaline hydrolysis to give 1-carboxymethyl-4-benzyloxycarbonyl-2-oxopiperazine (**32**) in one pot. The yield achieved was 35%.




**31** **32**

*Figure 4.7 Alkylation at N-1 by NaH activation*

This method was performed for **19** with little modification but only moderate success was achieved. Whilst **30** and **1a** were both identified by GCMS and high resolution LSIMS respectively their presence was only minor. Unreacted **19** was the major compound (ca. 35%) recovered from the single pot alkylation/ hydrolysis. Reasons for the poor alkylation result were difficult to formulate. The most likely cause was premature destruction of the NaH, leading to partial deprotonation of **19** and thus only limited alkylation. However, such a scenario would only be possible if solvents were not fully dried or the reaction was not performed under strictly anhydrous conditions. Great care was taken to ensure that both of these measures were implemented in all experiments.

As can be seen, alkylation of **19** at N-1 was partial at best, a result that placed a serious limitation on producing **1a** via this compound. In contrast, it has been shown that **10** is readily cyclised to give the desired carboxymethyl group at N-1 (section 4.2), thus negating the requirement for separate alkylation at this position. It is this key difference which selects Scheme II′ over I′ for preparation of **1a**. Other reasons in favour of Scheme II′ include :

- better documentation of reactions
- shorter route
- higher overall yield
- no requirement for specialist equipment (eg. Kugelröhr apparatus)
- strictly anhydrous conditions are not required

### *4.6.1 Future Development of Scheme I′*

The options of preparing **1a** via **19** have by no means been fully exhausted but were beyond the scope of this project. However, for production of **1a** on a small scale Scheme I′ would offer the advantage of significantly lower costs, despite the two extra steps. In the given case the shortest route with the best yields, that is Scheme II′, was considered to be more desirable than lower costs.

## 4.7 Scheme III

Referring to Scheme III (section 3.4.1), protected diethylenetriamine (**23**) was prepared in ca. 100% yield by treating diethylenetriamine with two equivalents of salicylaldehyde.[10] Reaction of **23** with an excess of ethyl chloroacetate followed by a simple work-up in $CH_2Cl_2$ led to **24** in good yield. It was found that **24** could be deprotected (hydrolysed) using far milder conditions than reported previously.[11] The sodium salt of **25** could be isolated by treatment with NaOH(aq), removal of water to dryness then reconstitution in dry methanol. However, a satisfactory elemental analysis and NMR spectrum were not obtained for **25**.

Attempts were then made to prepare **22** from **25** in one pot, without isolating **21**. Some limited success was achieved, in that a species with desired empirical formula $C_{10}H_{17}N_3O_5$ was confirmed using high resolution LSIMS. Furthermore, an amide CO signal indicative of **22** was identified by $^{13}C$ NMR analysis of the crude reaction mixture. However, analysis by HPLC showed **22** to be part of a

mixture of at least four related compounds that were poorly resolved (Figure 4.8). Whilst MS and NMR suggested cyclisation of **21** to **22** had occurred, HPLC indicated that the conversion was only partial. The majority of **21** was not cyclised.

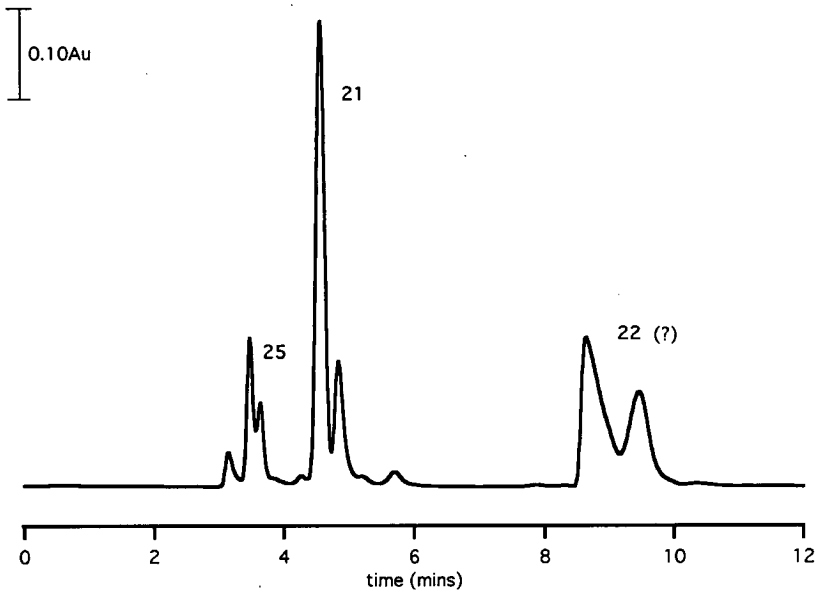



*Figure 4.8 Chromatogram of attempted conversion of **25** to **22** (via **21**). See section 7.4 for HPLC conditions.*

Further attempts to resolve the components of the cyclisation experiment were largely unsuccessful. However, in order to complete investigation of Scheme III the final step was performed using mixed product. Thus a sample of mixed **21/22** was treated with excess sodium chloroacetate to furnish a species with correct molecular formula $C_{12}H_{19}N_3O_7$ (by high res LSIMS). Aside from removing excess sodium chloroacetate from the reaction mixture no further work-up was done. Whilst there was some evidence to indicate Scheme III could generate **1a**, the approach suffered from several serious limitations, including :

- amino acid intermediates which were extremely difficult to separate/purify
- at least two extra steps compared with either Scheme I′ or II′
- reliance on the cyclisation of **21** to **22** (shown to be the limiting step)
- steps 5 onwards were largely untried, thus yields and other defining data were not known

These limitations precluded any further development of Scheme III. However it was of some interest to at least note that **21** may be converted to **22** in acidic media, analogous to the conversion between **10** and **5** (or **6** and **1b**). Scheme III could perhaps be improved if **21** was converted to the methyl ester of **22** using $SOCl_2$/ MeOH. Treatment of the methyl ester with ethyl chloroacetate (rather than sodium chloroacetate) would then provide an ester which when hydrolysed gave **1a**. Thus the formation of at least two troublesome amino acid intermediates could be avoided. In particular Scheme III showed us that it was best to avoid amino acid intermediates where possible. Thus the other schemes were developed with this in mind and incorporate the use of esters, converting to the amino acid **1a** only at the end.

## 4.8 Conclusions

At least three independent but related methods have been demonstrated for preparation of the DTPA cyclic degradation product. By discussing and comparing the merits of each method it has been possible to select Scheme II′ as the most suitable route to **1a**. Thus Scheme II′ has been applied to produce sufficient amounts of ligand **1a** for potentiometric titrations with selected metal ions.

## 4.9 References

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# CHAPTER 5

## Determination of Stability Constants : Techniques

### 5.1 Introduction

#### *5.1.1 Why Determine Stability Constants ?*

At the most fundamental level, a stability (or formation) constant provides a numerical description of the affinity of a ligand for a metal in solution. There are a variety of other reasons for calculating such constants, including :

- evaluation of the success of ligand design
- calculation of the concentration of the various species in solution
- calculation of other thermodynamic parameters, particularly enthalpies and entropies of complex formation

Due to the widespread use of organic ligands, such as EDTA and DTPA, in industries such as papermaking[1] and surfactants,[2] knowledge of their behaviour with metals under a particular set of conditions is vital. Stability constants can provide at least one indication of this behaviour. In the present study the aim will be to determine the environmental/ pulping process implications of **1a** by comparing the stabilities of **1a** complexes with those of DTPA. For example, if **1a** is found to have significant chelating ability, then the monitoring scheme used for DTPA might be extended to incorporate **1a**.

## 5.2 Techniques Available

There exists a wide variety of experimental techniques for the determination of formation constants, including potentiometry, spectrophotometry, polarography and NMR. Stability constants for DTPA have been determined most commonly by potentiometry[3] and also by spectrophotometry.[4] Attractive features of potentiometry include simplicity, rapid analyses, reliability and convenience. Whilst spectrophotometry is another possibility it is far more tedious and time consuming compared to potentiometry. It is an ideal method if a compound is only sparingly water soluble or if its acid dissociation constant lies outside the recommended range for potentiometry ($pK_a$ 2.0-11.0).[5] For the given cyclic DTPA degradation compound a potentiometric technique will be employed for the determination of stability constants, given the reasons above.

## 5.3 Stability Constants

Stability constants are the equilibrium constants for reactions between metal ions ($M^{x+}$) and ligands ($L^{y-}$) in aqueous solutions. Equilibria may be represented as

$$M^{x+} + nL^{y-} \rightleftharpoons ML_n^{(x-ny)+}$$

in which the role of the solvent and the charge on each species is ignored. The overall stability constant ($\beta$) at equilibrium is defined by convention as (ignoring charges)

$$\beta_n = \frac{[ML_n]}{[M][L]^n}$$

where square brackets are used to denote concentration and $\beta$ is the product of the stepwise formation constants $K_1$, $K_2$,...It should be stressed that all the coordination sites of M are occupied by water, so that the reaction simply involves replacement of one or molecules of water by L.

When L is a weak acid or base, hydrogen ions and metal ions compete for sites on the ligand. At low pH, such a ligand binds $H^+$ preferentially. For example, the overall reaction between glycine and $Cu^{2+}$ may be given as

$$2\,NH_3CH_2COO^- + Cu^{2+} \rightleftharpoons Cu(NH_2CH_2COO^-)_2 + 2\,H^+$$

Quantitative formation of $CuL_2$ occurs only if the liberated $H^+$ is removed by titration with standard alkali. Titration of the complexant in the presence of M leads to lower pH values than if L were titrated alone. This can be seen from Figure 5.1.

The difference between the curves shown in Figure 5.1 can, by rather lengthy calculation, reveal the stability constants. The majority of recorded stability constants (IUPAC) have been determined by such a titration method.[5] Versatility has been improved by the introduction of software to assist with data collection and processing.[6,7] Programs involved in the collection and processing of titration data will be discussed in section 5.5.

## 5.4 Experimental Procedure in Potentiometric Titrations

### *5.4.1 Preparation and Treatment of Materials*

It is important to ensure that all materials be of the highest possible purity. Precise measurements depend on good starting materials in addition to good measuring instrumentation and technique. The ligand should ideally be available in crystalline form and be recrystallised and characterised by elemental analysis and NMR prior to use. It may be prepared as a stock solution (0.01-0.05M) if stable or weighed into the reaction vessel for each run.[6]

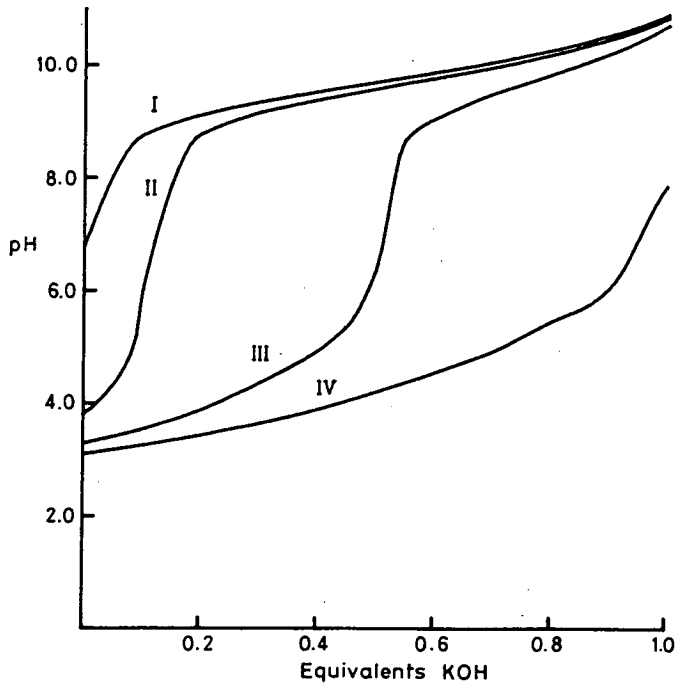



*Figure 5.1 Titrations of 0.02M glycine (gly) at T = 25 °C and μ = 0.15 M ($KNO_3$). Curve I 0.02M gly, Curve II 0.02M gly + 0.001M Cu(II), Curve III 0.02M gly + 0.005M Cu(II), Curve IV 0.02M gly + 0.01M Cu(II)*

Metal ions to be investigated can be prepared as standard solutions (0.02M) of their salts or again weighed directly into the reaction vessel.[6] Perchlorates and nitrates are common anions of choice, since they have large radii which discourage competition between L and counter-ion for a site on M. Easily hydrolysed metals require an excess of acid in the reaction solution to prevent partial precipitation.

Clearly it is sensible to use the same species as both counter ion and anion for the background electrolyte. For the bulk cation, a species with little or no tendency to form complexes with L is required, thus sodium and potassium are popular choices and their perchlorates are extremely water soluble.

Standard acid and base (eg. $HNO_3$, NaOH) should have similar concentration to the background electrolyte and furnish the same ionic species. Alkali must be carbonate free, as determined by a barium hydroxide test. Commercial volumetric solutions are usually carbonate free. All solutions containing hydroxides should be prepared with deionised water and standardised against potassium hydrogen phthalate. Solutions should be stored in polyethylene vessels protected with $CO_2$ adsorbents.

Stock solutions should be analysed by at least two independent methods.[6,8] Since the initial concentrations of L, M and H form the basis of all subsequent calculations, great care should be exercised with the initial analyses.

### *5.4.2 Apparatus*

Titrations are conveniently performed in a cell similar to the one depicted in Figure 5.2.[9] Temperature is regulated by the circulation of thermostated water through a double walled glass vessel. Rubber bungs or O-rings can be used to exclude contact with the atmosphere. Sufficient openings are provided in the cap to accommodate electrodes, gas lines and passage of materials into the cell.

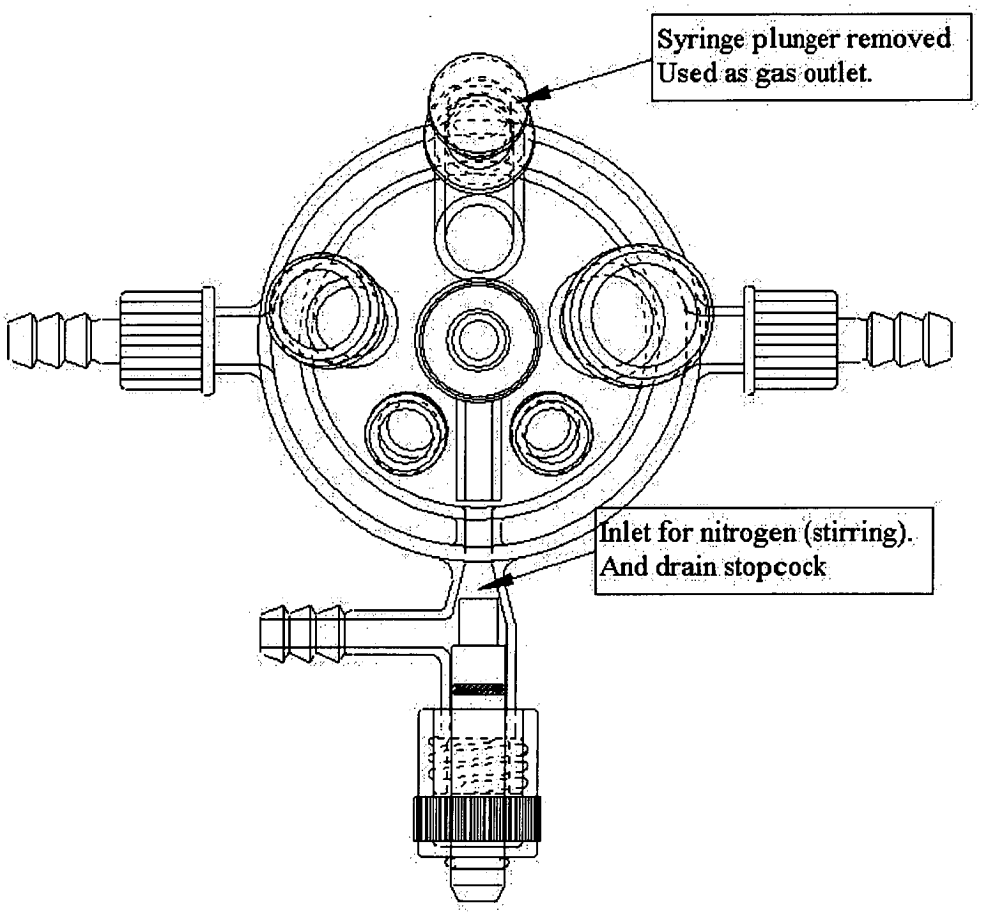
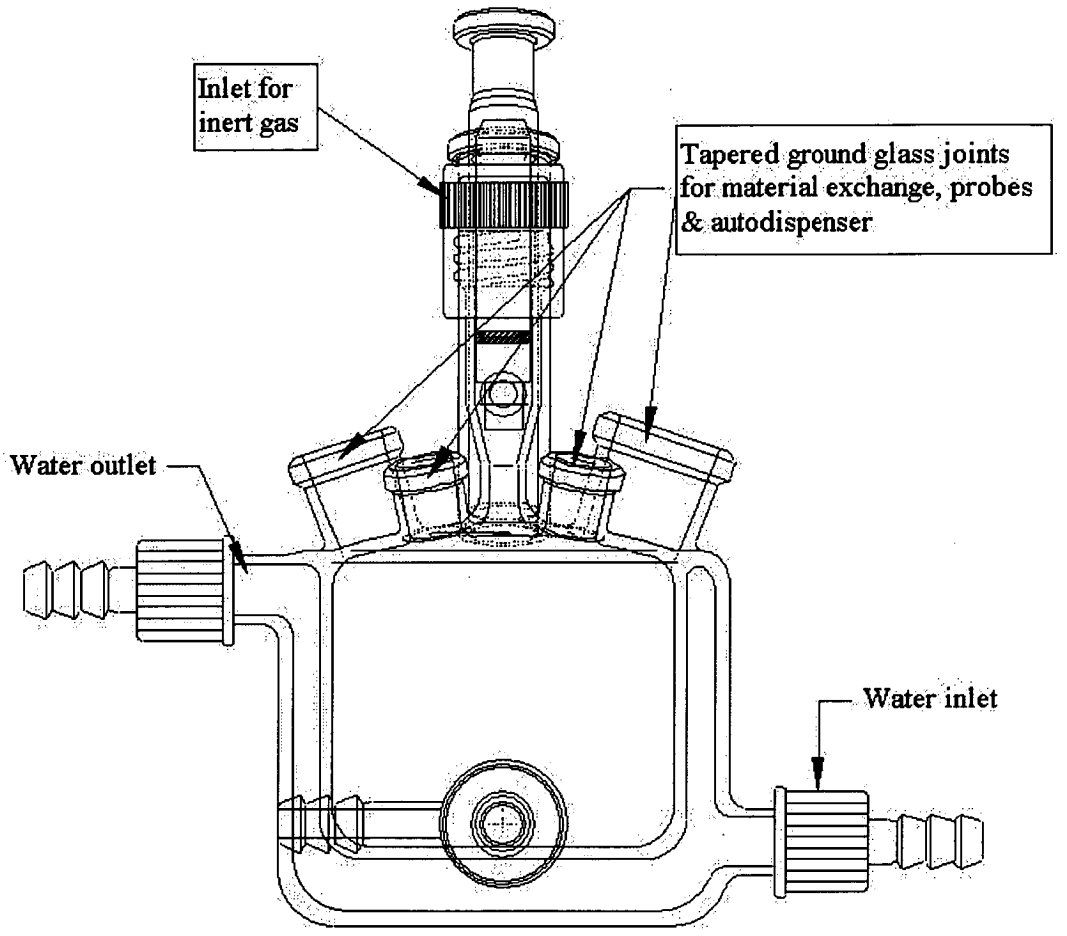



*Figure 5.2 (a) Plan view of custom made glass titration vessel*




**Side View of closed titration cell**

*Figure 5.2 (**b**) Side view of custom made glass titration vessel*

Agitation can be achieved either by use of a paddle or magnetic stirrer or a stream of inert gas. Standard base (or acid) is delivered via a capillary tip or dispenser just below the surface of the reaction solution and can be measured to 0.05 mL if necessary. A cell capacity of 70-100 mL is recommended in order that a volume of 50 mL of experimental solution can be contained.[5,6] The complete titration system can be automated and interfaced to a computer to facilitate data collection and analysis. Automatic or semi-automatic titration systems are finding widespread application in many areas and can produce extremely precise and accurate results.

### *5.4.3 Reaction Solution*

The reaction solution typically comprises precisely measured amounts of L, M, standard acid (if required) and sufficient background electrolyte to give the desired ionic strength (often 0.1M). Doubly distilled water is added to make up the final volume. If common practice is observed, then the reaction solution has low p[H] and contains the fully protonated form of the ligand.

### *5.4.4 Calibration of the Titration System*

Before a titration can be performed the titration system (ie pH probe) must be calibrated in terms of either pH or mV. Calibration with standard buffers[10,11] for which the $pK_a$'s are very accurately known under specified conditions is often used. Two or three point calibrations are possible with these buffer solutions, covering the expected pH range for the titration. The pH electrode may also be calibrated using a strong acid/base titration (Gran's method).[6] Another calibration method involves setting the pH meter to measure mV data which are then converted to pH using the Nernst equation. Thus calibration involves determination of system standard potential (E°).

Many types of autotitration apparatus deliver standard acid/base via a rotary reciprocating pump. These are set in the factory to dispense a standard volume (often 0.05 mL) per revolution but for best results a pump should be calibrated by

the researcher before use. For pump calibration a series of aliquots are dispensed, weighed, and the average weight used to calculate a calibration constant. An acceptable calibration constant should be between 0.900 and 1.100 with relative standard deviation <0.1%.

### *5.4.5 Typical Experimental Run*

As all future work relies on the thorough characterisation of the ligand, initial titrations are aimed at determining the acid dissociation constants, proton stoichiometry and formula weight of L. Thus an acidic solution of L is titrated against standard base such that a generous number of data points are obtained for each experimental run. Equilibrium, observed as a stable meter reading, must be established before addition of further base. It has been found that for most systems protonation and deprotonation are complete within the time required for mixing.[6,12] The titration curve (pH versus mL titrant) thus obtained for the ligand only is used to calculate $pK_a$ values.

In a subsequent run an aliquot of metal ion solution is added, plus sufficient acid to ensure L is fully protonated, then the solution is titrated as before.

It is important that at least two different mole ratios of M:L be evaluated, since the internal agreement of any one set of results is insufficient proof to discount competing equilibria. If identical profiles are obtained using different reactant mole ratios, then results are clearly reproducible and the formation of mixed or polynuclear complexes is negligible. Repetition of titrations is probably only necessary as one step in trouble-shooting if the pH profiles derived from two different runs on the same system do not correspond.[12]

## 5.5 Calculation of Stability Constants

### *5.5.1 Initial Analysis*

It is sensible to process any exploratory set of measurements at least by graphical means, such that a general view of the system behaviour may be obtained. In this way the presence of polynuclear or mixed complexes may be detected and one can learn much about mononuclear systems from the shape of the equilibrium curve (Figure 5.3).

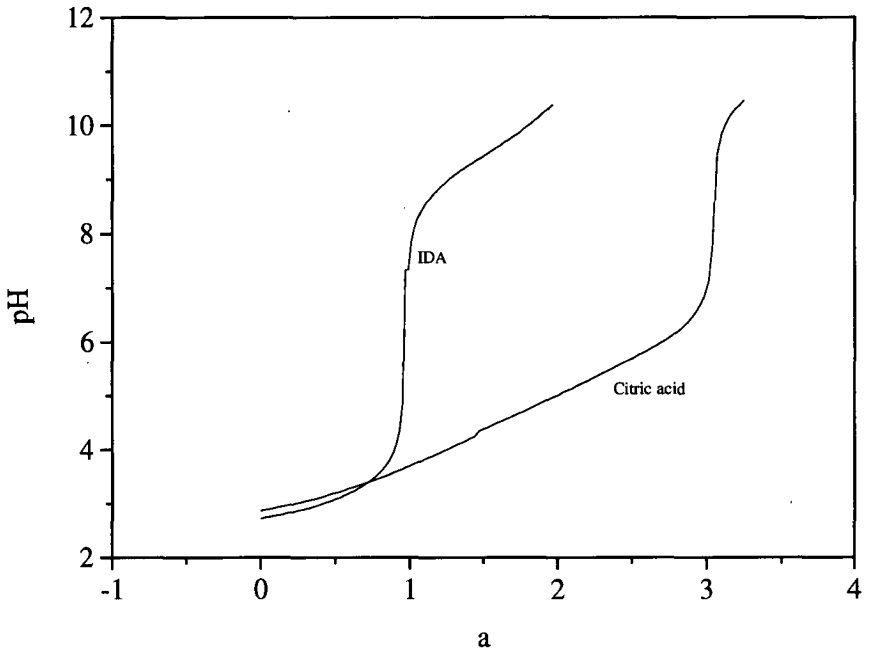



*Figure 5.3 Potentiometric equilibrium curves of IDA and citric acid at T = 25 °C, μ = 0.10 M ($NaClO_4$) and a = moles of base added per mole of ligand*

Plotting an equilibrium curve can also reveal suspect points which may, if shown to be invalid, be excluded from further analysis. Systematic errors can also be exposed, which must then be investigated and remedied. If preliminary graphical analysis suggests the data are free from obvious errors then definitive values for stability constants can be determined using computational methods.

### *5.5.2 Computational Methods*

The object of any computational method is to find the *best fit* between experimental and theoretical functions. If the data are not in question then the model (theoretical function) must be flawed. A poor model is either incomplete or fails to adequately describe the species present. Thus after verification of the experimental data the model may be altered one item at a time until acceptable agreement is obtained between the model and the experimental function.

The computational method adopted will vary with the type of system being studied; the best method for calculating the $pK_a$ for a monobasic acid is unlikely to be the most suitable for evaluating more complicated equilibria. However, the computational criteria are common to all types of systems.

An acceptable method will incorporate the following features :

- provide a clear indication of the complexes present
- make full use of the experimental data and be flexible to allow weighting of measurements
- locate systematic errors and allow corrections for them
- yield best values for parameters and give error limits
- be efficient in terms of time and effort

When final values for stability constants have been obtained, it is imperative that their nature and magnitude be compared with counterparts from previous systems. By doing so a disagreement between values can be detected and the system reinvestigated if necessary.

In the current investigation such a comparison will be limited by the fact that the system is under investigation for the first time. However the values obtained will be critically evaluated in terms of the principles and guidelines of coordination chemistry and the requirements for equilibrium constant determination.

It is possible to achieve a good least squares fit whilst the magnitude of the stability constant is far removed from reality.[6] This can arise from the relative ratios of $[ML_n]$ to [M] during the course of the titration. If the reaction is either substantially incomplete at the end of titration or complete at the start, then potentiometric data used for calculation of stability constants will incorporate significant error. Appreciable quantities of all equilibrating species must be present at once in order that accurate constants be determined. To discern whether reacting species are present in sufficient amounts (ie measurable) at equilibrium, it is useful to plot species distribution curves (Figure 5.4). Species distribution curves can also be helpful in testing the validity of published stability constants.

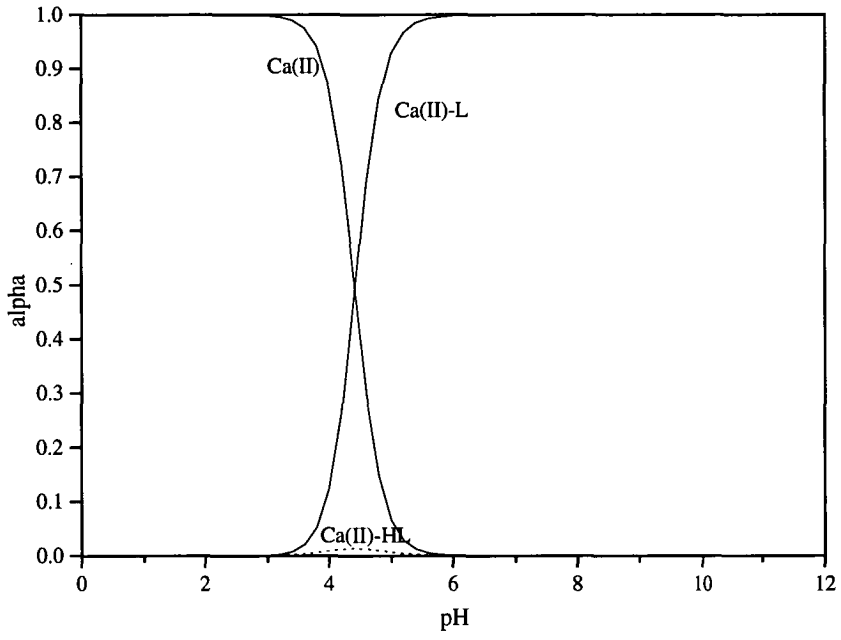



*Figure 5.4 Species distribution curves for 1 mM Ca(II)-EDTA system at 1:1; T = 25°C and μ = 0.10 M ($NaClO_4$)*

### *5.5.3 Structure of programs*

Programs for use in equilibrium analysis all have several parts, or blocks, in common. A typical program for computation of stability constants from titration data (mL base, pH) may consist of the following blocks :

1. INPUT. This block reads the data and may perform some preliminary calculations. Most programs require initial estimates for certain parameters, such as β values.
2. RESIDUAL SUM OF SQUARES. This block computes the standard deviation of fit (U) which is then minimised.
3. MINIMISATION. A search is made for the values of the parameters (eg. β's) involved in the minimisation of U. Many programs use a least squares method

combined with algorithmic processes.

4. ERROR ANALYSIS. This block calculates the confidence interval of the parameters, their standard deviations, correlation coefficients and other statistics.

5. GOODNESS-OF-FIT TEST. Here statistical methods are used to test the best models obtained from those put into the computer, giving a final model.

6. ADDITIONAL SUBROUTINES. Various mathematical subroutines are contained in this block.

7. OUTPUT. Values for parameters, with their statistics, are written here.

A more detailed discussion of program structure can be found in Meloun et al.[7]

### *5.5.4 Examples of programs*

There are many programs now available for the computation of equilibrium constants from potentiometric data; some examples are given Table 5.1. Two programs from Table 5.1 have been selected for further discussion.

*Table 5.1 Computer programs for the determination of equilibrium constants from potentiometric data*

| Program | Ref. |
|---|---|
| BEST | 6 |
| BSTAC | 13 |
| LOGMIN | 14 |
| MAXIPOT-F | 15 |
| MINIQUAD | 16 |
| MUCOMP | 17 |
| PKAS | 18 |
| PROTAF | 19 |
| SUPERQUAD | 20 |
| TITAN | 21 |
| TITFIT | 22 |

BEST[6] is an example of a FORTRAN program designed to calculate $pK_a$'s and stability constants from titration data. The basic algorithm in BEST aims to minimise the standard deviation of fit (SIGFIT, U) between observed and calculated pH values of the entire titration curve. The program essentially solves a series of simultaneous equations where some values of β are known (from previous calculations) and some are unknown. The initial pass of the program uses both known and estimated values of the unknown constants.

The algorithm in BEST is utilised in the following sequence :

1. Begin with a set of known and estimated overall stability constants (β's) and compute $[H^+]$ at all equilibrium points
2. Compute U and minimise
3. Adjust the unknown β's and continue iterations until no further minimisation of U can be achieved, thus providing the final β values

Fine-tuning is possible through minimisation in U by the variation of any of the input parameters which define the mathematical shape of the titration curve. The weighting factor used when computing U serves to increase the sensitivity of the computations in buffer regions and to lessen the influence of less accurate pH values near inflection points.

Another FORTRAN program for computation of stability constants, SUPERQUAD (SQ),[20] operates in a similar manner to BEST. SQ uses a nonlinear least squares curve-fitting algorithm for minimisation of U and determination of stability constants. As with BEST the user must provide initial estimations of any unknown β values which are then refined with repeated passes of the program. SQ is interactive, allowing the user to vary several input parameters for refinement of calculations. The authors of SQ have recently released a suite of programs,

HYPERQUAD (HQ),[23] which allow facilities for use of standard data files and standard procedures across data from a number of different sources, including potentiometric, spectrophotometric and NMR. The main difference between SQ and HQ is that HQ is able to deal with spectrophotometric data in addition to potentiometric data. The authors are currently developing a Windows version of HQ which they hope will eliminate the restrictions on experimental data (in terms of processing capability) and allow for the on-screen display of more information simultaneously using scrolling windows.

The algorithm(s) built into programs such as BEST and SQ assume that the data were obtained at equilibrium. If hydroxy species are important then equilibrium conditions will take some time to achieve. Thus it is vital to ensure that sufficient time is allowed to reach equilibrium such that programs have the most reliable data with which to work.

It is beyond the scope of this investigation to present a more detailed discussion of computational methods than that described above. Suitable graphical and computational treatments of pH measurements generated by potentiometric titrations may be found in Martell and Motekaitis[6] and Meloun et al.[7]

## 5.6 Common Sources of Error and Their Minimisation

There are many potential sources of error in the determination of stability constants by titrimetry. It is highly recommended that commercially pure ligands for which $pK_a$'s and stability constants are known (such as citric acid or EDTA) be investigated prior to any evaluation involving new ligands, such that an appreciation of the experimental method, equipment and any computational methods can be gained.[6,8]

### *5.6.1 Measurement Errors*

Poor data can arise from several sources, including improper calibration of the pH meter/ electrode system, problems with behaviour of the glass electrode, impure or otherwise improper characterisation of the ligand and undesirable side reactions/ phase separations that alter quantities of L, M and H during the course of the titration.

### *5.6.2 Care of Electrodes*

The pH or ion-selective probe, when not in use, should be stored by immersing the bulb in saturated KCl. Occasionally the glass electrode may exhibit erratic behaviour following contact with heavy metal ions. Treatment of the probe with dilute HCl for a short period can restore normal function.

Failure of the reference electrode is possible if KCl crystallises in the opening which serves as the liquid junction to the experimental solution. Removal of such blockages restores normal probe function. The liquid junction potential may also be altered due to the flow of experimental solution back into the reference electrode. Keeping the reference electrode filled and avoiding immersion above the filling hole are steps which prevent backflow of experimental solution and thus minimise undesirable changes to liquid junction potential.

### *5.6.3 Reagents*

Poor standardisation of the reagents can lead to serious error and must be prevented. For example, a small concentration error for a pentaprotonated ligand (eg. DTPA) would result in a five-fold error in the amount of available $H^+$. Whilst this may not significantly alter equilibrium constants for major species, a large deviation could be introduced into the calculations for constants of minor species.

Stock solutions of the less basic metals should contain excess acid to prevent partial hydrolysis and subsequent precipitation of hydroxides.

Other precautions which should be observed include maintenance of an inert atmosphere (Ar or N) and careful control of temperature and ionic strength.

### *5.6.4 Temperature*

Temperature can be the most common source of error in pH measurements. The slope of the pH electrode changes with temperature; modern electrodes automatically compensate for this effect. For potentiometric titrations performed at constant temperature thermal effects are not usually a problem.

Temperature may also bring about changes in chemical equilibrium. Calibrating the electrode with well-characterised buffers, for which pH values are known at different temperatures, minimises undesirable impact on chemical equilibria.

### *5.6.5 Titration Errors*

A common observation encountered when titrating with alkali is for the values (in the set of $pK_a$ values) to show an upward trend during the course of the titration. This is usually due to the effect of an impurity, which reduces the actual amount of ligand present. Water is by far the most common impurity.[5] Thus all ligands submitted for determination of dissociation constants should be of analytical purity and dried thoroughly.

Upward trending pH may also signal excessive stirring, causing loss of solution to the reaction cell walls. Furthermore, the calculated amount of ligand may be present but not entirely dissolved, thus making it impossible to obtain satisfactory data. An incongruous set of results is often indicative of decomposition during the titration.

## 5.7 Equilibrium Measurements

Equilibrium conditions must be achieved prior to any pH measurements. Two general criteria apply; that the electrode be given sufficient time to adjust to the new conditions and that restrictions due to the kinetics of complex formation/ dissociation be considered. The first criterion is usually satisfied within 30 sec provided the stirring rate is adequate for homogeneous mixing.[6] The boundaries for the second criterion are usually far broader.

When reaction kinetics are not limiting then sluggish response time may be attributed to either a spent electrode or one contaminated, for example, by repeated exposure to polyvalent metal ions. A spent electrode may show slow drift for hours whilst a contaminated probe stops drifting. Spent electrodes are not restored by treatment with dilute acid whereas a poisoned probe can be rejuvenated by following the recommendations of the manufacturer.

The rate of chemical equilibrium clearly depends on the system under investigation. Suspicion of slow kinetics may be confirmed by allowing longer equilibration time during a repeat experiment. A titration becomes impractical if the run time exceeds 12 h since it is difficult to assure system calibration for such an extended period.[6] Additional problems associated with long equilibration time include contamination of the experimental solution (electrolyte from the reference electrode), undesirable side reactions and phase separation. At least two approaches can be employed to address slow reaction kinetics; successive data points can be obtained from a number of fresh experimental solutions, or by a batch method, where solutions representing successive data points are prepared, stored for the appropriate time then assayed and reassayed to deduce the equilibrium pH value.[6]

## 5.8 Matrix Effects on Stability Constants in Real Solutions

The stability constants determined for **1a** with selected metal ions in vitro will represent "ideal" values. The composition of the experimental solution is well defined in terms of the reactant species (M, L and H) and conditions are carefully controlled to optimise determinations. In contrast, the real solution (eg. pulping effluent sample) has quite an undefined composition and M-L equilibria will be a function of many components, not restricted to pH only. Thus it is likely that complex formation in real samples will deviate somewhat from formation under strictly controlled laboratory conditions, leading to differences in the magnitude of stability constants.

Stability constants calculated for **1a** by the described potentiometric method could be ranked, in order of decreasing magnitude. This ranking could then be assumed to hold in the case of real solutions, though values may be elevated or depressed in relation to the "ideal" values. This is of course a rather gross over-simplification but may help our understanding of complexing power of **1a** in pulping liquors.

It may ultimately be necessary to thoroughly characterise the real solution then determine how matrix components affect complex formation. Complete compositional characterisation of a real pulping liquor is envisaged to be extremely tedious and time consuming. It may also be that complex formation in real samples depends only on a few key components, though many may have to be screened to locate these. In any event the determination of stability constants for real samples will be a major task, far beyond the scope of this investigation.

## 5.9 Stability Constants of Aminopolycarboxylic Acids

As indicated in section 5.5.2, the magnitude of dissociation constants for **1a** will be compared with those of similar aminopolycarboxylic acids. A comprehensive library of critical stability constants of many ligand types has been compiled by

Martell and Smith.[24] These volumes have been recently rescrutinised and are now available as a database from the National Institute of Standards and Technology (NIST) web site (http://www.nist.gov/srd/webguide/nist46/46guide.htm).[25] According to the developers of the on-line database, whilst some new determinations involving EDTA type ligands are being performed, little or nothing new really emerges.[26]

Equilibria data for a number of aminopolycarboxylic acids with similar characteristics to **1a** are presented in Table 5.2. Note that Table 5.2 includes equilibria data for the structural isomer of **1a** (**1b**). Since **1a** and **1b** differ only at the position of the ring carbonyl one could expect titrimetry of **1a** to furnish similar equilibria data to **1b**. However it should be noted that the dissociation constants for **1b** were determined in 1968[27] and there has been no confirmation of these values by another study.

*Table 5.2 Protonation constants at T = 25°C and μ = 0.10M in aqueous solution*

| Ligand | $\log K_1$ | $\log K_2^H$ | $\log K_3^H$ | $\log K_4^H$ | $\log K_5^H$ | ref. |
|---|---|---|---|---|---|---|
| EAMA | 9.69 | 6.56 | 2.72 | 2.10 | | 28 |
| EDTA | 10.17 | 6.11 | 2.68 | 2.10 | 1.5 | 29 |
| DTPA | 10.45 | 6.11 | 2.68 | 2.0 | 1.5 | 29 |
| HEIDA | 8.66 | 2.20 | | | | 29 |
| IDA | 9.34 | 2.61 | | | | 29 |
| S-KP | 6.55 | | | | | 30 |
| **1b** | 8.40 | 3.52 | 2.7 | | | 27 |

## 5.10 Summary

Several different techniques for the determination of stability constants have been presented. From this discussion potentiometry has been selected as the most appropriate method for ligand **1a**. A thorough discussion of experimental procedure, with particular reference to equilibrium measurements and computation of titration data, has been given. It should be re-emphasized that the magnitude of the equilibrium constants calculated for **1a** will be evaluated with reference to those of other similar ligands.

## 5.11 References

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# CHAPTER 6

## Determination of Stability Constants : by Experiment

### 6.1 Introduction

In Chapter 5 potentiometry was identified as the most appropriate technique for determination of stability constants of aminopolycarboxylic acid type ligands. However during an exploration of the solution behaviour of **1a** the opportunity to utilise NMR for determination of equilibrium constants arose. NMR is being applied more frequently for the determination of equilibrium constants of compounds of biological interest including amino acids,[1] peptides[2] and antibiotics.[3] NMR measurements offer a particular advantage over potential measurements (ie potentiometry) because they can be used to monitor the chemical shift (as a function of pH) of a particular compound in a mixture which may often contain other acids and bases. Information on chemical shift versus pH obtained in this way is independent of the presence of protolytic "impurities" provided there is no direct interaction between the latter and the compound being monitored. That this is a major advantage can be seen by recalling the importance in potentiometry of excluding $CO_2$ from reaction solutions (sections 5.4.2 & 5.6.3).

It should be recognised that NMR measurements require an amount of experimental time that is highly dependent on concentration of the species, the nucleus being monitored and the pulse sequence employed. Managing these factors properly is essential, especially if a species shows poor NMR sensitivity or changes/ degrades with time.

This chapter describes how both potentiometry and NMR were employed to determine equilibrium constants and pH responses of ligand **1a** in aqueous solution. An evaluation of the magnitude of stability constants has been made with reference to other similar ligands.

## 6.2 Materials

All chemicals were obtained from commercial sources and were of analytical purity. Ligands **1a** and **5** were prepared as outlined in Chapter 7, section 7.5 and characterised by NMR, MS and microanalysis.

## 6.3 General Procedure (Potentiometric and NMR Analyses)

A custom made glass titration vessel[4] (section 5.4.2, Figure 5.2a & b) with sufficient openings for pH electrode (Orion, model 9157), thermocouple, autodispenser, stirrer (propeller blade), and gas flow was utilised. Temperature was maintained at 25°C ±0.1°C by circulation of thermostated water through the jacketed reaction vessel. Atmospheric oxygen and carbon dioxide were excluded by maintaining a slight positive pressure of either nitrogen or argon in the titration cell.

Potentiometric titrations were performed using an Orion 960 autochemistry system interfaced to a computer to facilitate data collection and analysis. The system was calibrated using prepared buffers (NIST buffers) and strong acid/base titration. A calibration was performed prior to each titration.

All solutions were prepared using Milli-Q water. Alkali solutions (0.10 M) were prepared from commercial semi-conductor grade NaOH and stored in PE bottles protected from carbon dioxide. Ligand stock solutions (100mL) were made from thoroughly dried material and stored to exclude light and the atmosphere.

Solutions of metal perchlorates were prepared from AR grade material and stored in PE bottles protected from the atmosphere. Solution concentrations of ligand and metal were in the order of 10-20 mM. Alkali solutions were standardised with weighed quantities of dried potassium hydrogen phthalate. The exact endpoint of this and other subsequent titrations was determined by the first derivative method.

For a typical run, a weighed amount of sodium perchlorate was added directly to the reaction vessel, followed by the calculated volumes of ligand and metal perchlorate solutions. The reaction solution was then made up to volume with Milli-Q water. The reaction solution was stirred under an inert nitrogen (or argon) atmosphere and allowed to equilibrate for about 30 min. Successive pH measurements were then made after each addition of small (0.05 or 0.10 mL) increments of standard base until the pH reached about 10.5-11.5. Timed measurements were made. The tip of the autodispenser was immersed in the reaction solution throughout the titration and stirring was continuous. The protonation constants were determined from at least duplicate potentiometric titrations and the stability constants from two titrations where the ratio M:L differed.

For NMR determinations a typical potentiometric titration (as described above) was monitored as follows. Briefly, between 0.3-0.5 mL was withdrawn by plastic syringe from the reaction solution at known pH and analysed by a presaturation water suppression $^1$H NMR technique (Chapter 7, section 7.8.1). Care was taken to exclude $CO_2$ from sample solutions in NMR tubes and each 0.3-0.5 mL sample was returned to the main reaction solution after NMR analysis. Typical NMR analysis time per experimental point was 5-10 mins. Up to 20 such data points were collected in this manner starting at low pH (2) and finishing at pH ~11. Assignments of **1a** $^1$H resonances were made possible by using a gradient enhanced COSY pulse sequence (Chapter 7, section 7.8.1) at some 6-8 arbitrary pH values during the course of the first NMR monitored titration. Several $^1$H

NMR titrations were performed to evaluate reproducibility.

## 6.4 Calculations

Protonation constants and metal stability constants were calculated using programs SQ (Chapter 5, section 5.5.4) and HYPNMR[5] Program SQ was first available in 1985 but remains essentially unchanged as part of the recently released HQ suite of programs.[6] Program HYPNMR, also part of the HQ package, calculates $pK_a$'s from chemical shift data in a manner similar to how SQ calculates $pK_a$'s from potentiometric data. Further details concerning how the program operates, including the application of the Gauss-Newtown-Marquardt method in the refinement are available elsewhere.[5]

The suitability of SQ and HYPNMR for equilibrium constant determinations was established using data generated from titrations of commercially sourced ligands whose dissociation constants are well known (Table 6.1). The good agreement achieved between reference and experimental values also indicated a robust experimental method.

Other programs considered were PKAS[7] and BEST.[7] Program PKAS proved useful for determination of protonation constants but was not designed for computation of stability constants. Both $pK_a$'s and stability constants could be calculated using BEST but this software was difficult to use in comparison to SQ.

*Table 6.1 Protonation constants at T = 25°C and μ = 0.10M ($NaClO_4$) in aqueous solution by programs SQ and HYPNMR*

| Ligand | $logK_1$ | $logK_2^H$ | $logK_3^H$ | ref. |
|---|---|---|---|---|
| Citric acid | 5.69 | 4.35 | 2.87 | 8 |
| | 5.71 | 4.39 | 2.80 | this work (SQ) |
| | 5.65 | 4.34 | 2.76 | this work (HYPNMR) |
| IDA | 9.34 | 2.61 | | 9 |
| | 9.25 | 2.64 | | this work |
| S-KP | 6.55 | | | 10 |
| | 6.53 | | | this work |

## 6.5 Uncertainties

For each SQ data file the user must provide estimates of error for the parameters titre volume(mL) and pH (or mV). For the given Orion system these were determined to be 0.01mL and 0.02 pH units respectively. These values were incorporated into every SQ data file. No such error estimates were required for HYPNMR.

Where four $pK_a$'s were incorporated in SQ metal-ligand data files the statistical measures (chi-squared, sigma) appeared quite elevated (eg. 150, 6). By omitting the $pK_a$ associated with the greatest deviation ($pK_a$ 2.5) and recalculating, the statistical measures showed marked improvement and the stability order/ values (section 6.8) remained essentially unchanged. Thus the stability constants listed in Table 6.3 have been calculated using $pK_a$'s 10.5, 8.3 and 3.5 in SQ metal-ligand data files.

For the limited HYPNMR determinations chi-squared (a measure of confidence) was typically 50 or less and sigma (measure of fit) between 1-4. For SQ determinations chi-squared was found consistently to be below 80, with the exception of two (larger) results and sigma was consistently in the range 4-6, also

with the exception of two (larger) results. Similar ranges for these statistics were observed from the SQ output files of commercial ligands IDA and citric acid. Whilst these measures of fit appear quite large, the comparison of $pK_a$ values in Table 6.1 shows that reliable results could still be achieved. The measures of fit are also considered reasonable given the limitations of the experimental apparatus and method. For example, a number of researchers recommend pH data be measured to three decimal places. This was not possible with the system used in this work. The presence of a small percentage of an impurity (section 6.6.1) may also have had an influence on measures of fit, especially in the case of SQ determinations. Another possible limitation of the system relates to the computational model used in SQ. Whilst every effort was made to accommodate likely species in the model it is quite possible that the exclusion of some species could affect the output (log betas).

The estimated error (deviation) associated with each experimental log beta was found usually to be 0.10 for ML and MHL complexes (eg. $\log\beta_{FeL}$ 16.3 ± 0.10) and 0.20 for $ML_2$ complexes. Stability data taken from other sources are cited without estimated deviations but are considered reliable within ±1 unit of the last significant figure shown.

## 6.6 Results and Discussion

Representative data/ output files (SQ/ HYPNMR) may be found in Appendix A at htttp://www.chem.utas.edu.au/students/damienb.html. All files appear in their original form and can be downloaded if required. File descriptions are provided and users hot-link directly to each file.

### *6.6.1 Initial Titration* (Ligand Only)

In order to demonstrate that **1a** was not decomposed during the course of a titration, an acidic solution of **1a** was titrated with 0.10 M NaOH to pH 11 then to

pH 2 with 0.10 M $HClO_4$. The forward/ reverse potentiometric titrations were also monitored by $^1H$ NMR (refer section 6.3). As can be seen from Figure 6.1, the titration curves coincide with each other within experimental error, provided the dilution effect is taken into consideration. The $^1H$ NMR spectra of the titrations showed that **1a** species formed during the forward (initial low pH) titration matched those **1a** species detected in the reverse titration. Furthermore, several alkaline (8-12) solutions of **1a** were re-analysed by $^1H$ NMR after 24hr and could be shown to contain the characteristic resonances for **1a**. Together these observations suggested that no decomposition of **1a** occurred during the titration.

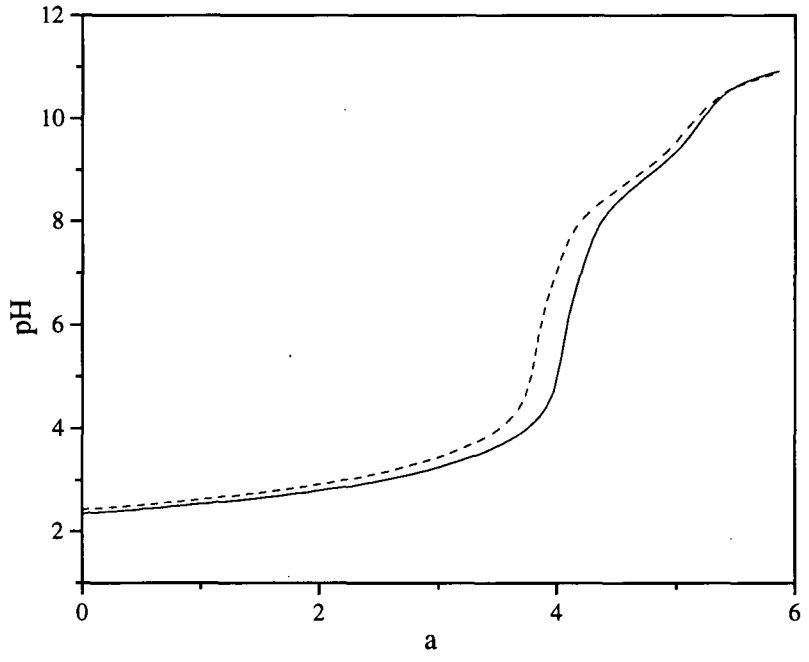


*Figure 6.1 Titration of ligand* ***1a*** *at T = 25°C and μ = 0.10 M ($NaClO_4$) in aqueous conditions ------* ***1a*** *+ 0.10 M $HClO_4$; ——* ***1a*** *+ 0.10 M NaOH*

Examination of the initial titration using SQ revealed a misfit between experimental and calculated curves in the pH range 6.5-7.5. One possible explanation for this is that **1a** contained an impurity, despite evidence to the contrary (eg. satisfactory elemental analysis). Decomposition had been ruled out but the possibility of rearrangement remained. The most likely rearrangement of **1a** would involve transition between chair and boat conformers. However, such behaviour was not considered likely to produce a misfit between experimental and calculated potentiometric curves.

To further explore the likelihood that **1a** contained an impurity, more thorough investigation of a typical potentiometric titration using $^1$H NMR was made. The $^1$H spectra were processed using linear prediction and combined to generate the profiles indicated in Figures 6.2 and 6.3. The profile shown in Figure 6.3 is a ~ x10 enlargement of the region where the presence and action of an impurity (U) can be observed. The impurity profile has been produced by subtracting ligand (L) resonances from each $^1$H NMR spectrum. This gives many of the peaks a "clipped" appearance at the base and also leads to the disappearance of some peaks. All resonances, aside from L, are due to the impurity.

Whilst the identity of the impurity could not be established (due to limitations in the sensitivity of the instrument) it is clear from Figure 6.3 that U was a coupled system (multiplet at pH 4.25) and several of the peaks tracked L resonances during the course of the titration. This led to the conclusion that U was most likely related closely to L, perhaps a partially hydrolysed form of the tri ester **27**. The level of impurity appeared to remain constant during the course of a titration, as indicated by $^1$H NMR. Steps were taken to remove the impurity (Chapter 7, section 7.6) from ligand **1a** prior to any further titration work but ultimately the purity could not be improved beyond ~95%.

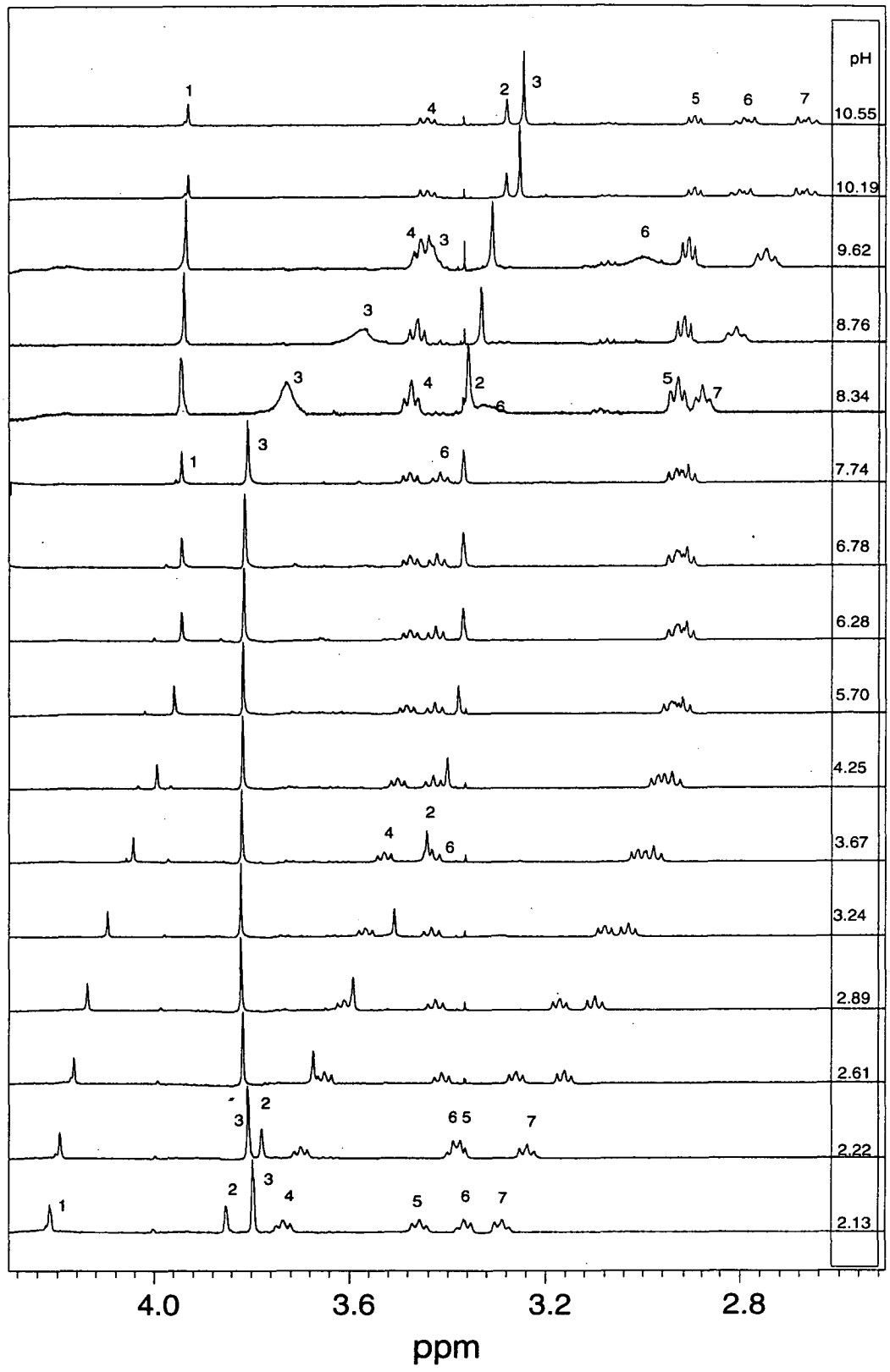


*Figure 6.2* $^{1}H$ *NMR titration profile of ligand* **1a** *only. Peak resonances denoted 1-7.*

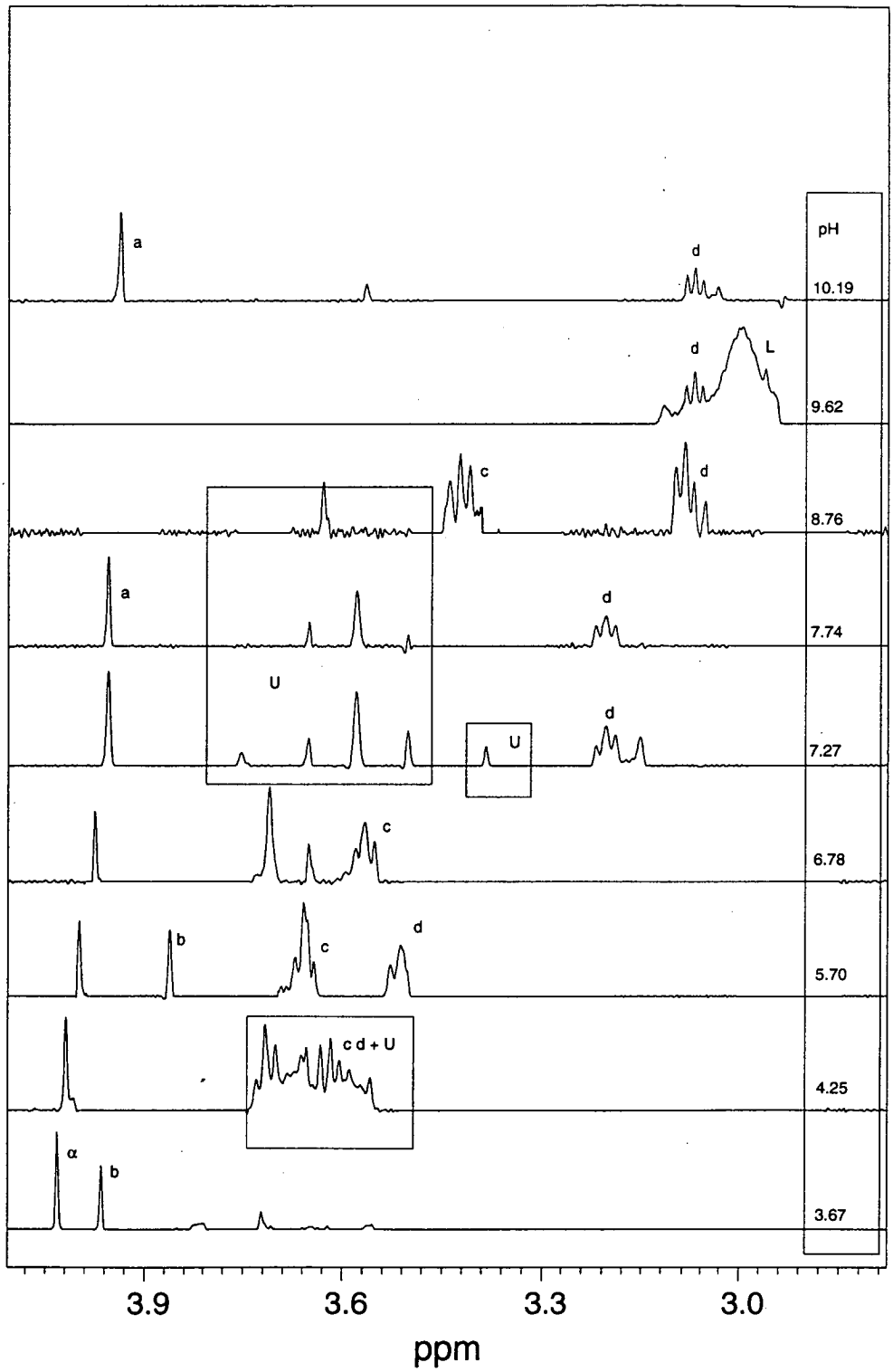


*Figure 6.3* $^{1}H$ *NMR titration profile of impurity (U) in ligand (L)* ***1a***

## 6.7 Protonation Constants (by $^1$H NMR Titrations)

The determination of $pK_a$'s of ligand **1a** was explored initially using NMR, given that this technique is better equipped than potentiometry to handle the presence of extraneous materials.[5] Of the five dissociation constants predicted for ligand **1a**, three could be ascertained using HYPNMR. The log protonation constants determined from chemical shift data are 8.5, 3.0 and 2.0 (Table 6.2). The $pK_a$ values obtained for **1a** are listed in Table 6.2, together with $pK_a$ values reported previously for related ligands.

*Table 6.2 Protonation constants of **1a** and related ligands at T = 25°C and μ = 0.10M ($NaClO_4$) in aqueous solution*

| Ligand | $logK_1$ | $logK_2^H$ | $logK_3^H$ | $logK_4^H$ | $logK_5^H$ | ref. |
|---|---|---|---|---|---|---|
| **1a** | 8.5 | 3.0 | 2.0 | | | this work |
| **1b** | 8.40 | 3.52 | 2.7 | | | 11 |
| EAMA | 9.69 | 6.56 | 2.72 | 2.10 | | 12 |
| EDTA | 10.17 | 6.11 | 2.68 | 2.10 | 1.5 | 9 |
| DTPA | 10.45 | 6.11 | 2.68 | 2.0 | 1.5 | 9 |
| IDA | 9.34 | 2.61 | | | | |
| U-EDDA | 10.9 | 5.53 | | | | 9 |
| U-KP | 5.51 | | | | | 10 |
| S-KP | 6.55 | | | | | 10 |

The $^1$H NMR titrations of **1a** also allowed some general observations of the protonation sequence. Ligand **1a** has seven chemically nonequivalent sets of protons that give rise to seven distinct NMR signals (Figure 6.2). The seven resonances were assigned as 1-7 respectively (Figure 6.4) based on several gradient COSY experiments. In general, deprotonation of the ligand causes chemical shifts to move upfield, most likely due to shielding of proximal $^1$H nuclei as oxygen electrons become more localised.[13,14]

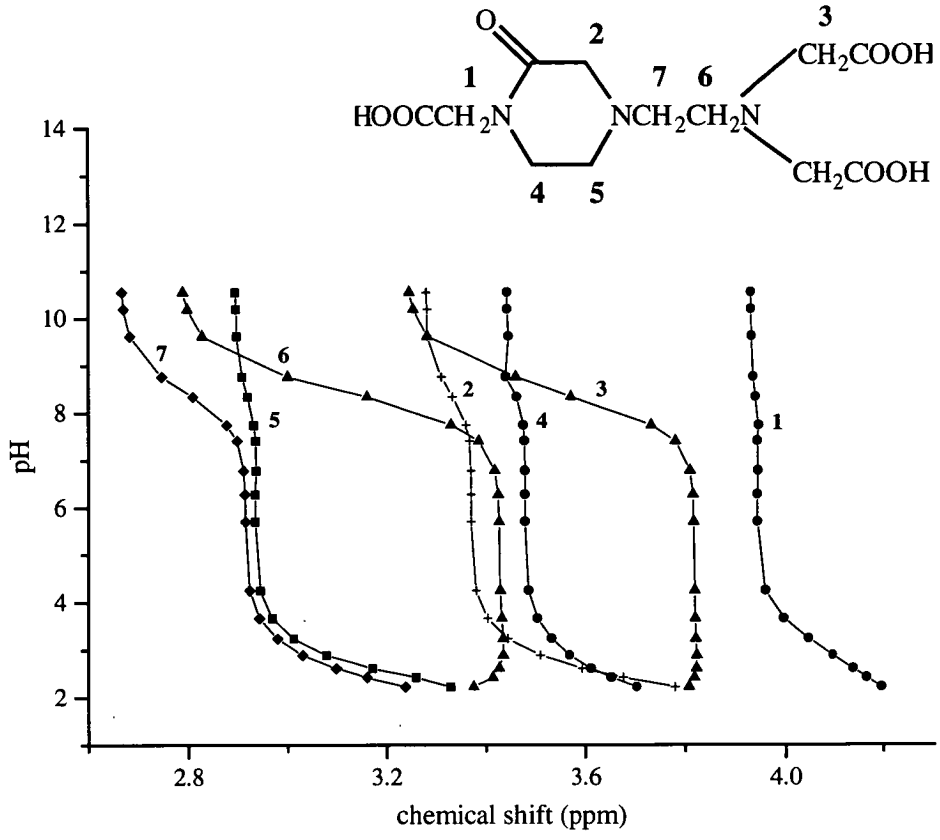


*Figure 6.4 pH dependencies of the $^1H$ NMR chemical shifts in aqueous* **1a** *solution at T = 25°C and μ = 0.10M ($NaClO_4$). Numbers 1-7 represent $^1H$ resonances*

It is clear from Figure 6.4 that two sets of curves are related (1,4 and 3,6), given that each pair exhibits similar slope. The upfield movement of resonances 1 and 4 between pH 2.5-4 suggests that initial deprotonation of **1a** is not associated with the diacetate portion of the molecule. Further addition of base had no major effect until pH 8.5-10 when resonances 3 and 6 (lesser extent 7 >2 >5 >4) move sharply

upfield, suggesting deprotonation at the diacetate end of the molecule. Beyond pH 10 there is essentially no change to resonances 1, 2, 4, 5 suggesting that deprotonation associated with the ring was most likely complete. However it can be seen that resonances 3 and 6 continue to trend upfield, perhaps suggesting that another $pK_a$ may lie above pH 10.

Since it was not possible to determine all the $pK_a$'s of **1a** using HYPNMR it is a little difficult to make any further inferences other than to state that initial deprotonation appears to occur at the monoacetate portion of the molecule, followed by deprotonation at the diacetate end.

The third protonation constant ($pK_3 = 2.0$) is not obvious from Figure 6.4. If the second protonation constant ($pK_2 = 3.0$) is associated with the monoacetate group (Figure 6.4) then it seems likely that $pK_1 = 8.5$ and $pK_3 = 2.0$ are related to the diacetate region of the molecule. Further weight can be added to this argument given that IDA and N-(2-hydroxyethyl)iminodiacetic acid (HIDA) have $pK_a$'s of 2.6/9.3 and 2.2/8.7 respectively. A possible dissociation scheme for **1a** based on the $^1H$ NMR and potentiometric titrations is given in Figure 6.5.

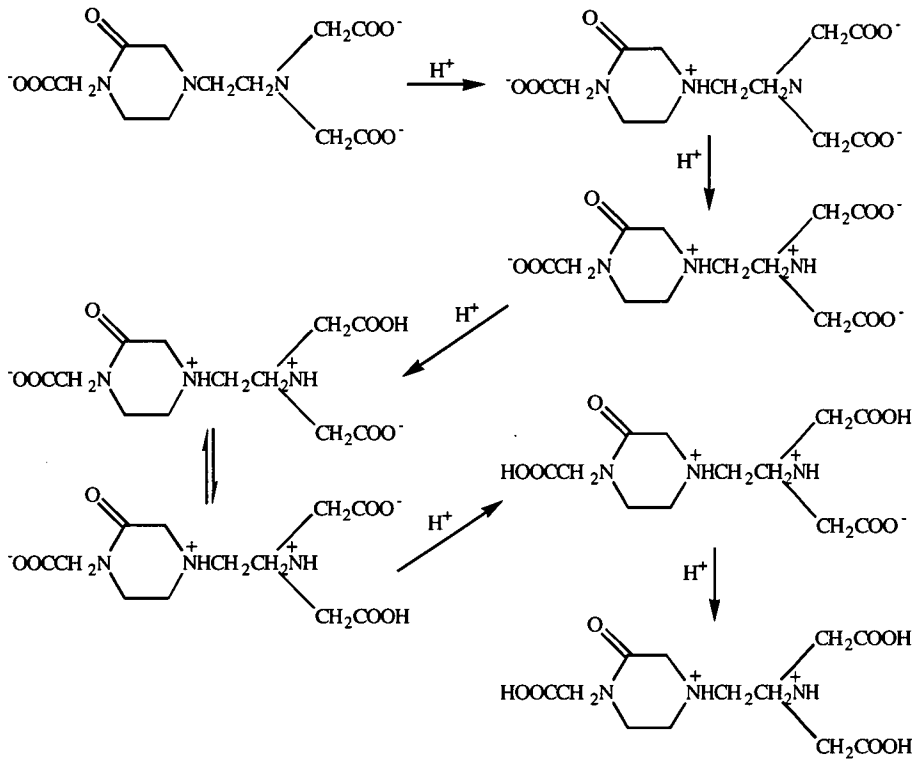

*Figure 6.5 Proposed protonation sequence of* ***1a***

Figure 6.2 provides a few further points of note regarding the protonation sequence. The narrow line width of resonance 3 (methylenes adjacent to diacetate) up to pH ~8 suggests either a rapid exchange equilibrium or that the diacetate sites are equivalent (Figure 6.6). After pH ~8.5 the diacetate sites remained equivalent on the NMR timescale but the exchange rate was much slower (Figure 6.7), giving rise to line broadening of resonance 3. The signal sharpened in response to an increase in temperature. The observation that resonance 1 (methylene adjacent to monoacetate) experienced no broadening as pH was varied was further evidence to suggest that the slow step (at pH ~8.5) is related to some form of exchange equilibrium involving both groups of the diacetate.

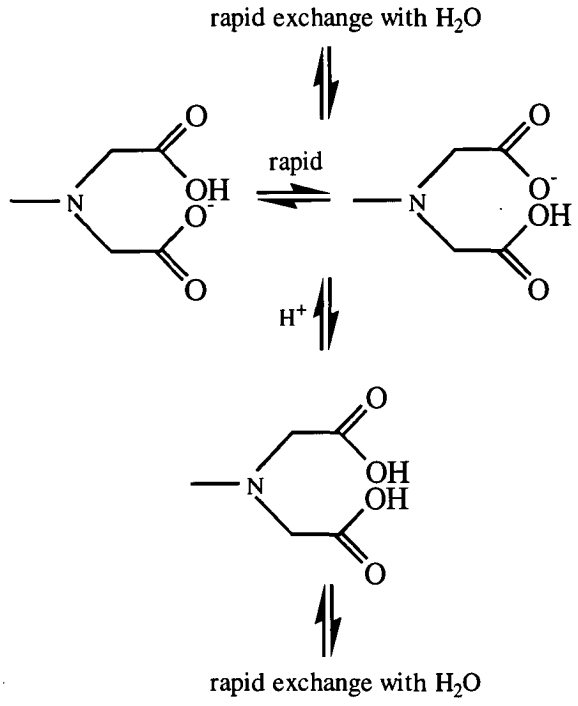


*Figure 6.6 Possible $H^+$ exchange equilibria for diacetate groups of* ***1a*** *(pH < 8)*

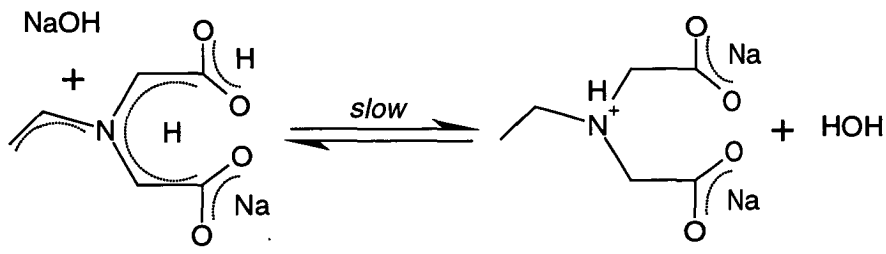


*Figure 6.7 Possible equilibria for diacetate groups of* ***1a*** *(pH > 8)*

The three protonation constants obtained for **1a** using HYPNMR are compared with those of analogous ligands (Table 6.2). The $pK_a$'s show most similarity to those of the structural isomer **1b**. This is not unexpected, given that the two molecules differ at only one position; the position of the ring carbonyl group has sufficient influence over solution behaviour to produce slightly different acid dissociation constants.

## 6.8 Stability Constants (by $^1$H NMR Titrations)

The introduction of a metal (Zn or Hg) into the titration system caused substantial broadening of $^1$H resonances (Figure 6.8) which made reliable assignments of chemical shifts impossible. The $^1$H NMR spectrum (Figure 6.8) at pH 10.3 displayed the *minimum* peak broadening for solutions pH 2-10.3. The NMR probe temperature was increased to ~50°C but no improvement to the broadening was observed. The responses of the remaining metals, expected to be analogous to (or worse than) both Zn and Hg, were not investigated.

Had there been more time available an appropriate method for metals would have been pursued. Such a method may have involved simplifying the chemical model used in HYPNMR. For example, by selecting fewer, reliable resonances that still define the entire ligand, the model may be sufficiently defined for stability determinations. Work with model compounds (EDTA, IDA) could assist greatly with method development. For example, use of model compounds would help conserve the scarce quantities of the synthesised ligand, plus stability constants determined by HYPNMR could be compared with known values. For some metals, such as Mn(II), it will never be possible to calculate stability constants by NMR due to their paramagnetic nature.

In conclusion the robust nature of the NMR technique (citric acid and **1a** titrations) and the applicability of the processing program HYPNMR has been demonstrated. It would also seem feasible that the technique could be successfully

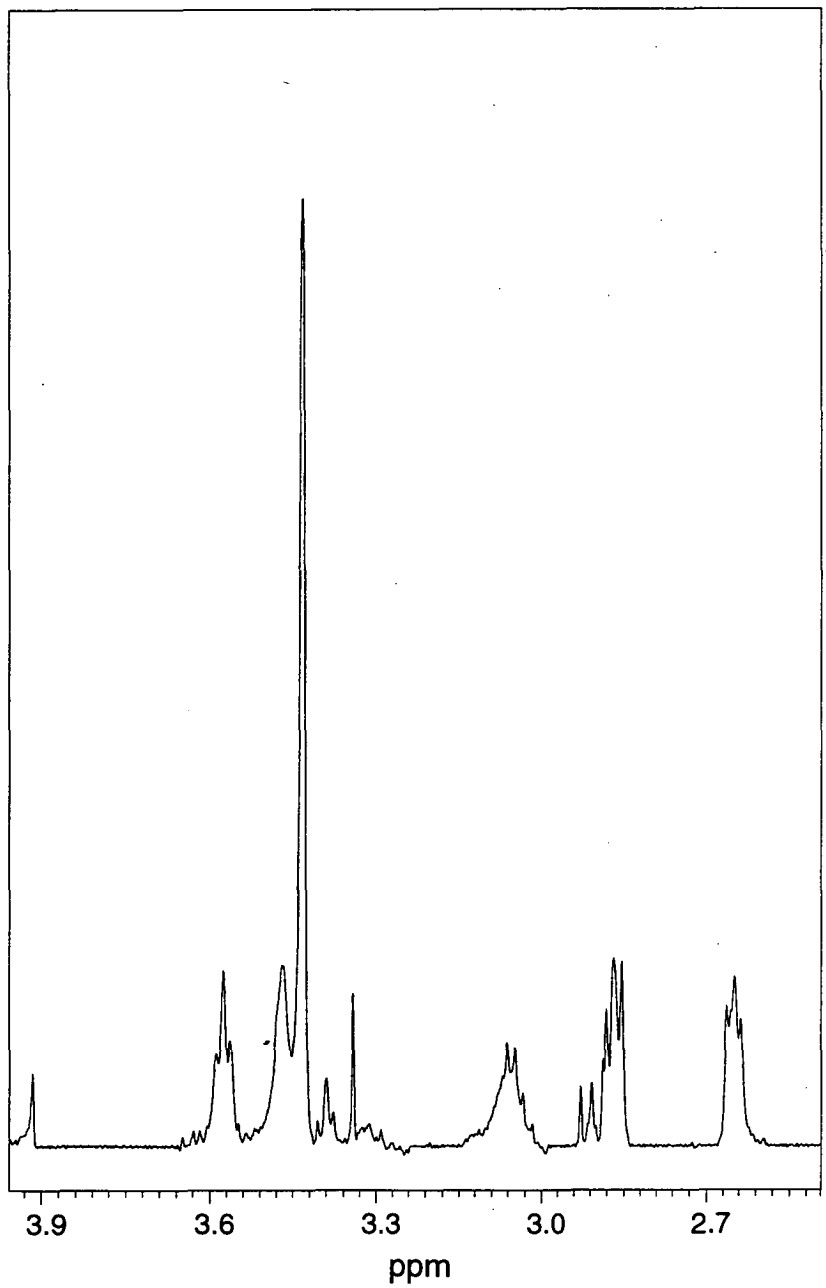


*Figure 6.8* $^1H$ *NMR spectrum of 2.5 mM* ***1a*** *+ 2.6 mM Zn(II) solution at pH 10.3 (T = 25 °C, μ = 0.10 M* $NaClO_4$*)*

developed to enable stability constant determinations.

## 6.9 Protonation Constants (by Potentiometric Titrations)

It was possible to determine four protonation constants from potentiometric data (Table 6.3). As can be seen from Table 6.3 the $pK_a$'s found by the two different techniques do not fully coincide, a not unusual result given that (HYP)NMR was expected to better cope with the presence of any extraneous material (section 6.1).

*Table 6.3 Comparison of log protonation constants for* ***1a*** *at T = 25 °C and μ = 0.10M ($NaClO_4$) in aqueous solution by $^1H$ NMR and potentiometry*

| $logK_1$ | $logK_2^H$ | $logK_3^H$ | $logK_4^H$ | ref. |
|---|---|---|---|---|
| | 8.5 | 3.0 | 2.0 | this work (HYPNMR) |
| 10.5 | 8.3 | 3.5 | 2.5 | this work (SQ) |

The general agreement between $pK_a$ values derived from SQ and HYPNMR was encouraging and had more data points been collected it may have been possible to locate another $pK_a$ near 10.5 using HYPNMR. For consistency and validity the protonation constants determined by SQ were used for SQ calculations of stability constants rather than $pK_a$'s found by HYPNMR.

## 6.10 Stability Constants (by Potentiometric Titrations)

The potentiometric equilibrium curves for the formation of divalent and trivalent metal chelates of ligand **1a** are shown in Figures 6.9 and 6.10 respectively. It should be noted that in general one titration was performed per day, with a calibration between titrations. Results for two different mole ratios of M:L are shown although four different ratios were explored. The reason for this is as follows; at M:L of 1:5 similar results to 1:1.5 and 1:2 were obtained but as expected measures of fit and estimated deviations were far greater. At higher M:L of 2:1, mass balance was compromised by formation of precipitates at intermediate pH.

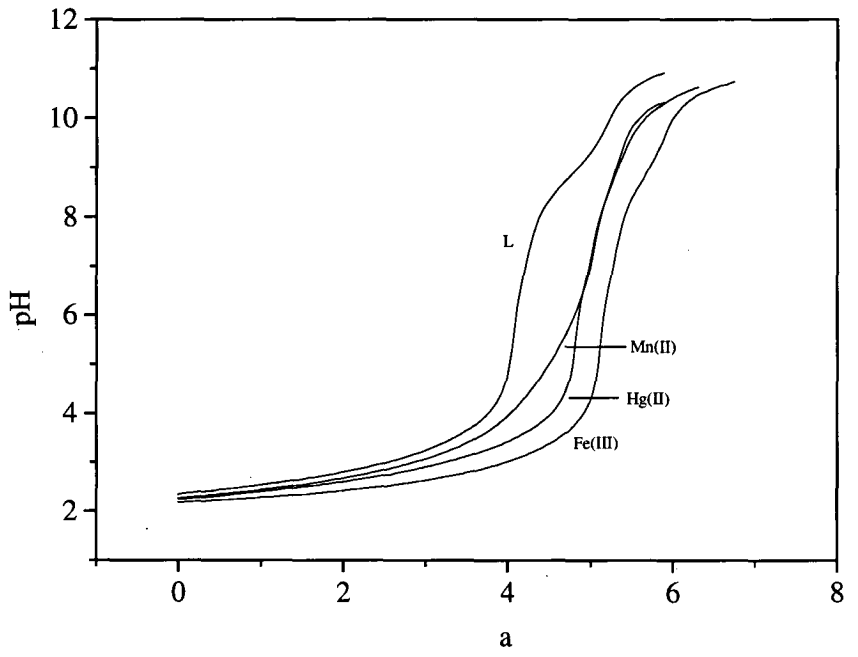


*Figure 6.9 Potentiometric equilibrium curves of 16 mM* ***1a*** *and* ***1a*** *complexes with metal ions (as indicated); T = 25 °C and μ = 0.10 M ($NaClO_4$), M:L 1:1.5*

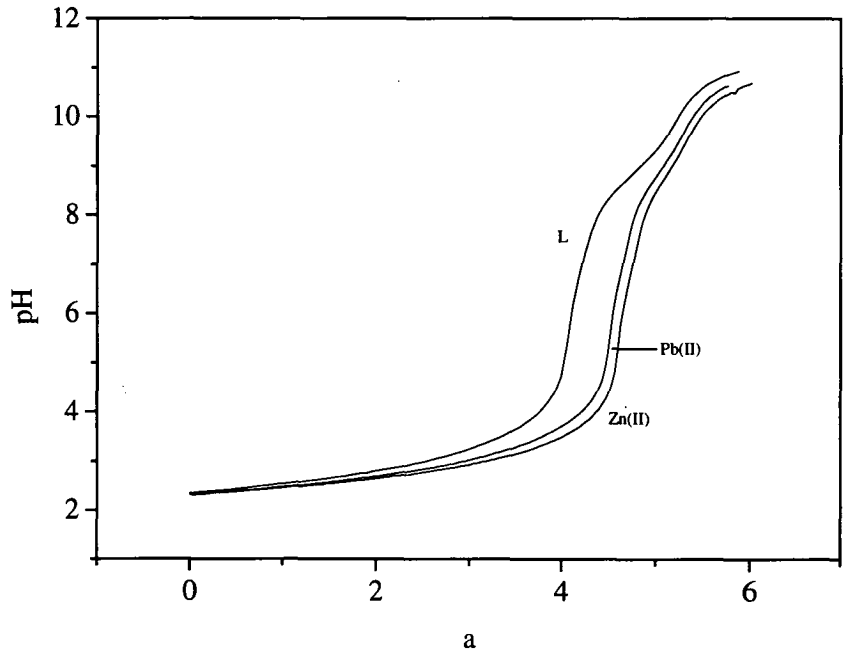


*Figure 6.10 Potentiometric equilibrium curves of 16 mM* **1a** *and* **1a** *complexes with metal ions (as indicated); T = 25 °C and μ = 0.10 M ($NaClO_4$), M:L 1:2*

Initial analysis of the curves in Figs. 6.9 and 6.10 indicate reasonably strong chelate formation with all the metals investigated, with the possible exception of Mn(II). The relative order of chelation tendencies can also be discerned, the lower the pH of the initial buffer zone, the greater the stability of the metal chelate. From an examination of Figs. 6.9 and 6.10 the stability sequence appears to be Fe(III) > Zn(II) > Hg(II) ≅ Pb(II) > Mn(II).

Analysis of the data by program SQ shows that **1a** forms mononuclear (1:1) complexes with all the metals under consideration. All form monoprotonated chelates (MHL) and binuclear ($ML_2$) complex species also appear possible. No evidence for $M_2L$ complexes was found and hydroxo complexes were not supported by the model.

The stability constants (shown for divalent metals) were found to be consistent with the following chemical equilibria :

$$M^{2+} + L^{3-} \rightleftharpoons ML^{-} \qquad K_{ML} = [ML^{-}]/[M^{2+}][L^{3-}] \qquad (1)$$

$$ML^{-} + H^{+} \rightleftharpoons MHL \qquad K^{H}_{MHL} = [MHL]/[ML][H^{+}] \qquad (2)$$

$$ML^{-} + L^{3-} \rightleftharpoons ML_2^{4-} \qquad K_{ML_2} = [ML_2^{4-}]/[ML^{-}][L^{3-}] \qquad (3)$$

The stability constants defined by equations 1-3 are presented in Table 6.4.

*Table 6.4 Logarithms of stability constants of metal chelates of* **1a** *at T = 25°C and μ = 0.10 M ($NaClO_4$)*

| | ratio | metal ion | | | | |
|---|---|---|---|---|---|---|
| | M:L | $Mn^{2+}$ | $Pb^{2+}$ | $Hg^{2+}$ | $Zn^{2+}$ | $Fe^{3+}$ |
| $\log K_{ML}$ | 1:1.5 | 12.7 | - | 14.9 | - | 16.3 |
| | 1:2 | 12.5 | 15.1 | 15.1 | 15.6 | 16.6 |
| $\log\beta_{MHL}$ | 1:1.5 | 18.4 | - | 19.4 | - | 20.1 |
| | 1:2 | 18.2 | 18.9 | 19.4 | 19.1 | 20.0 |
| $\log\beta_{ML.L}$ | 1:1.5 | 16.4 | - | 19.1 | - | 20.8 |
| | 1:2 | 16.2 | 19.1 | 19.2 | 19.6 | 20.7 |

The stability order discerned from Figs. 6.9 and 6.10 was confirmed by numerical analysis using SQ and is quite consistent with orders observed for chelates of other similar ligands.[9] As shown in Table 6.4 there are no log stability constant values for Pb(II) and Zn(II) at M:L 1:1.5. Despite several titrations at this ratio for both metals, consistent results from SQ could not be obtained. However, based on the good agreement between 1:1.5 and 1:2 values for other metal chelates one could assume the 1:2 results for Pb(II) and Zn(II) would be a fair estimation of their stabilities.

As expected, the values in Table 6.4 show that the chelating ability of **1a** is significantly less than the parent compound DTPA. The chelating ability of **1a**, in terms of the five metals investigated, is more akin to NTA for Hg(II) and Fe(III) complexes and EDTA for Zn(II), Pb(II) and Mn(II) complexes (Table 6.5).

*Table 6.5 Log $K_{ML}$ values of metal chelates of* **1a** *and similar ligands at T = 25°C and μ = 0.10 M ($NaClO_4$)*

| ligand | LogK$_{ML}$ | | | | | ref. |
|---|---|---|---|---|---|---|
| | $Mn^{2+}$ | $Pb^{2+}$ | $Hg^{2+}$ | $Zn^{2+}$ | $Fe^{3+}$ | |
| **1a** | 12.6 | 15.1 | 15.0 | 15.6 | 16.5 | this work |
| EDDA | 7.0 | 10.6 | - | 11.1 | - | 9 |
| EDTA | 13.8 | 17.9 | 21.5 | 16.4 | 25.0 | 9 |
| DTPA | 15.5 | 18.7 | 26.4 | 18.3 | 28.0 | 9 |
| NTA | 7.5 | 11.3 | 14.6 | 10.7 | 15.9 | 9 |

The log stability constants obtained for $ML_2$ complexes (Table 6.4) appear low in relation to values for similar ligands, where $ML_2$ values are usually double those for ML. A clear reason for the apparent low $ML_2$ results is difficult to formulate, but it is possible that error in ligand concentration was the likely cause.

From work with physical models it appears sterically possible for all three carboxyl groups of **1a** to coordinate with either a divalent or trivalent metal. The complex so formed could exhibit appreciable stability due to the high number of atoms (at least 15) participating in ring formation. It also seems likely that the ring C=O group could participate in chelate formation though to a lesser extent than any of the carboxyl groups. More definitive data from modelling (molecular mechanics) or X-ray studies would be required to address any further points about complex structure.

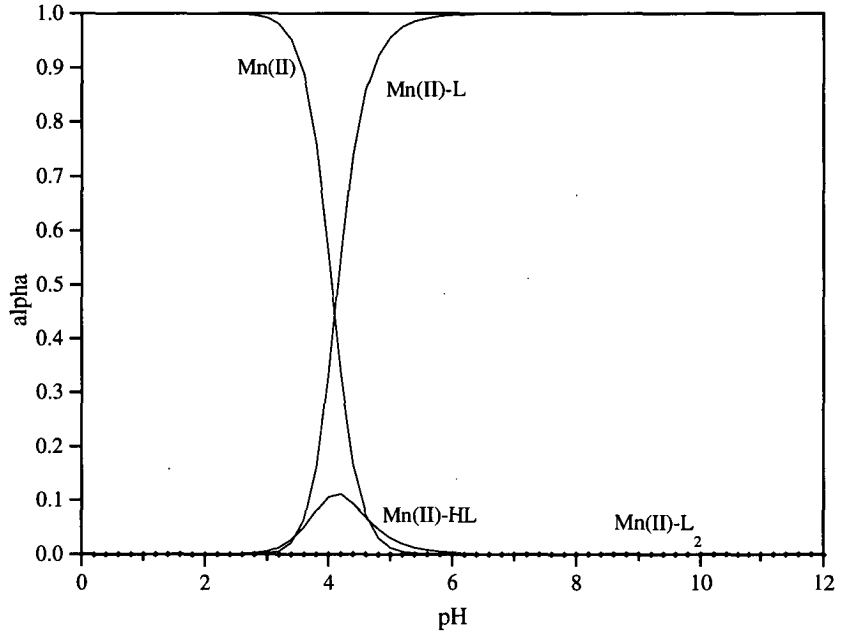


*Figure 6.11 Species distribution curves for 3 mM Mn(II)-**1a** system containing a 1:1.5 mole ratio of Mn(II) to **1a**. T = 25°C and μ = 0.10 M ($NaClO_4$)*

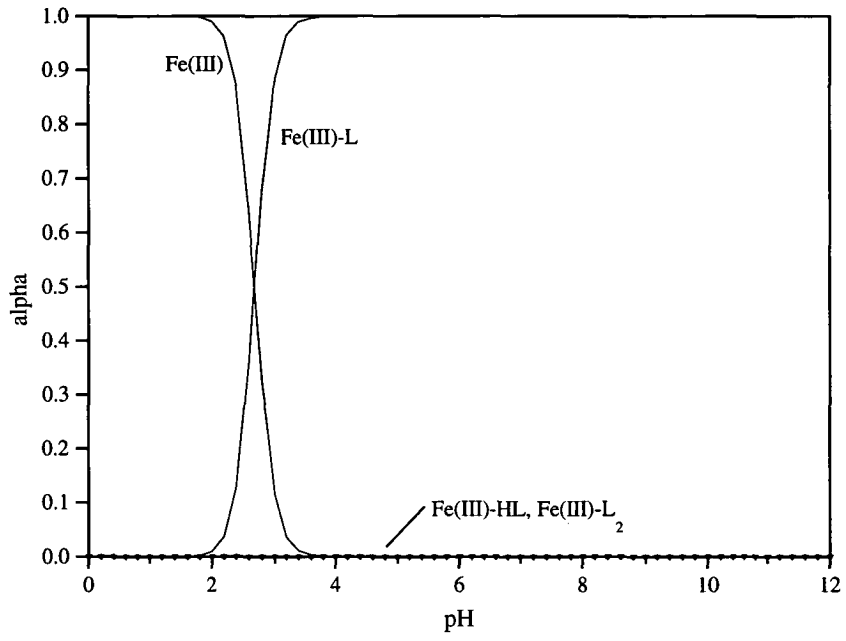


*Figure 6.12 Species distribution curves for 3 mM Fe(III)-**1a** system containing a 1:1.5 mole ratio of Fe(III) to **1a**. T = 25°C and μ = 0.10 M (NaClO4)*

As representative examples, species distribution curves (Figs. 6.11 and 6.12) were constructed for the most (Fe) and least (Mn) stable metal complexes. As shown, the ML complexes predominate in solution above pH 4 for Mn(II) and pH 3 for Fe(III). The protonated form of the Mn(II) complex shows a maximum concentration of about 10% whilst for Fe(III) the MHL complex is absent at the given concentration of L. The absence of the binuclear $ML_2$ species for both Mn(II) and Fe(III) (and implied for the remaining metals) may suggest that its concentration is not accurately known under the given conditions, or its existence is in some doubt. As noted (section 6.5), the greatest uncertainty was also associated with $ML_2$ results.

## 6.11 Implications for the Mill and Aquatic Environment

It is clear from the preceding discussion that **1a** possesses significant complexing ability. Therefore a monitoring program to determine the level of **1a** in pulping liquors seems an appropriate response. The remaining quantity of **1a** from this study could be used as a standard. Implementation would be relatively straightforward given that **1a** can be detected as the ferric complex by HPLC using the method developed for monitoring DTPA.[15] Based on previous monitoring work,[16] the level of **1a** in pulping effluent is anticipated to be less than that of DTPA, namely 5 ppm.

Of the five metals (Table 6.4) investigated in this study, Mn(II) has been implicated as having the greatest potential for decompostion of both $H_2O_2$ and DTPA in the pulping process.[17] However, as the stability of Mn(II)-**1a** is not very different from Mn(II)-DTPA (Table 6.5), then even if DTPA degrades, protection of $H_2O_2$ via Mn(II)-**1a** complexation would still occur. Another issue of some concern is the influence of **1a** on removal of heavy metals during wastewater treatment, since DTPA exerts some effect on the precipitation of Zn(II) and Mn(II) at this stage.[18] The influence is not anticipated to be very significant however, due to the likely low level of **1a** in pulping liquors.

Perhaps the greatest environmental impact of **1a** may be its effect on aquatic organisms, especially since it shows a tendency to persist in aqueous solutions over a wide pH range. It has also displayed good temperature and light stability. Studies of DTPA toxicity toward sensitive indicator organisms[19,20] *Daphnia carinata* and *Melanotaenia fluviatilis* suggest that at the levels present in mill discharge DTPA would pose little harm to aquatic life. A similar outcome might therefore be expected for **1a**. Furthermore, treated mill effluent is now being re-routed for irrigation, so the aquatic impact of **1a** should be negligible. The use of treated effluent (containing **1a**) for irrigation may in fact be slightly beneficial,

given that chelating agents are used for micronutrient fertilisation in hydroculture and soil application.

## 6.12 Summary and Conclusions

Protonation constants for ligand **1a** have been determined by two different techniques, namely $^1$H NMR titration and potentiometric titration. The $pK_a$ results showed general agreement, given that each model handles the presence of extraneous material differently. The utility of the NMR technique has been demonstrated and a method to facilitate NMR measurement of metal solutions could be developed in future.

Stability constants of **1a** with five metals were calculated from potentiometric data and follow the general trend observed for similar ligands. It should be reiterated (Chapter 1) that the discrete stability values found by SQ are less important than the observed trend of stabilities. Whilst the anticipated reduction in complexing ability compared to DTPA was observed, **1a** metal complexes had sufficiently high stability to justify further attention (eg. establishment of a monitoring program). The results have few implications for papermaking with regard to process changes although it may be necessary to evaluate the influence of **1a** on metal removal during wastewater treatment.

The major outcome of the complexing studies is that the use of DTPA as a chelating agent in thermomechanical pulping at ANM Albury is appropriate given the significant reduction in complexing power and concentration of DTPA degradation products such as **1a** in pulping liquors.

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# CHAPTER 7

# Experimental

## 7.1 General Procedures

NMR spectra were measured using a Unity-Inova 400 MHz instrument at normal probe temperatures unless otherwise indicated. Standard $^{1}H$, $^{13}C$, HETCOR, $^{13}CDEPT$, gHMQC and gHMQB experiments were performed to facilitate unambiguous assignment of resonances. Chemical shifts are expressed in ppm on the $\delta$ scale; tetramethylsilane (TMS) was used as an internal standard except for solutions in deuterium oxide, where tetramethylsilane propyl sulfonic acid (TMSPSA, Na salt) was used. Peaks are reported as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m). All MS data were collected with a Kratos Concept (Mach 3 software) mass spectrometer. GCMS analyses were performed using a Hewlett Packard 5790 Mass Selective Detector coupled to a HP 5890 GC fitted with a HP-1 (25m x 0.32mm i.d., 0.52μm film thickness) column. UV data were measured using a Shimadzu UV-160 UV-visible recording spectrometer and IR spectra on a Brüker IFS 66 FTIR spectrometer. Melting points were determined with a Gallenkamp melting point apparatus in open capillary tubes and are uncorrected. Elemental analyses were performed using a CHNS-O EA1108 (Carlo Erba Instruments) in the Central Science Laboratory, School of Chemistry, University of Tasmania.

Where anhydrous conditions were required all glassware and solvents were dried[1] and transfers made via gas-tight syringes.

## 7.2 Materials

### *7.2.1 For Chromatography*

Compounds **1a**, **5**, **7**, **17**, **19** and **27** were prepared according to the methods outlined in Section **7.5**. Their purity was established by NMR, MS and microanalysis. Acetonitrile (HPLC grade) was sourced from Merck. All other reagents were of analytical grade and used without further purification.

### *7.2.2 For Organic Preparations*

All chemicals used in the given syntheses were obtained from commercial sources and were of analytical grade. Where required, solvents were dried according to standard methods.[1] Silica gel used for column chromatography was flash grade (Kieselgel 60). For TLC, precoated silica gel (60 $F_{254}$) aluminium sheets were used. Samples of **6** (Akzo Nobel, Finland), **7** (University of Limoges/ National University of Mexico) and **21** (Akzo Nobel, Finland) were obtained and used without further purification.

## 7.3 Experimental for Chapter Four

## 7.4 Chromatography

### *7.4.1 Liquid Chromatograph Instrumentation*

For compound **1a** the liquid chromatograph was a Varian 9010 fitted with a Rheodyne 7126 injection valve (100μL loop) and a Varian 9060 Polychron photodiode array detector operating at 258nm. The analytical column was an Alltima 5μm $C_{18}$ (250 x 4mm). Samples were injected using a Varian 90100 autosampler and data processed with a Varian Star data system. Separations were performed isocratically at 2.0 mL/ min and ambient temperature.

For compounds **5** and **7** the liquid chromatograph consisted of a Waters 600 Multisolvent Delivery system equipped with a Waters 486 detector operating at 214nm interfaced with a data station running Maxima software. The analytical column (250 x 4.6mm) was an Activon Goldpak 5μm $C_{18}$. Separations were performed isocratically at 1.0 mL/ min and ambient temperature.

### *7.4.2 Mobile Phases*

For the trihydrochloride salt of **1a** a 4 mM octylamine solution (2000 mL) containing 8% MeCN was prepared and the pH adjusted to 6 using 2M $H_2SO_4$. A mobile phase comprising 82% octylamine solution and 18% MeCN was used to effect elution of Fe(III)-**1a**.

The mobile phase for **5** was prepared by mixing together 5mM octylamine (1850 mL) and MeCN (150 mL). After adjusting the pH to 7 with acetic acid the mobile phase was filtered and degassed before use.

The mobile phase for **7** was prepared by mixing together a solution containing ammonium acetate/pentanesulfonic acid (Na salt) both at 5 mM (1800 mL) and MeCN (200 mL). After adjusting the pH to 4.7 with a few drops of acetic acid, the mobile phase was filtered (0.45μm) and degassed prior to use.

### *7.4.3 Preparation and Analysis of Reaction Solutions by HPLC*

A few mg of the trihydrochloride salt of **1a** was dissolved in 2 mL of 0.1M $FeCl_3$ then buffered to pH 7 with phosphate buffer after standing for 15 min. No filtering was necessary.

All samples of **5** and **7** (standards and alkylation solutions) were prepared in deionised $H_2O$ and injected at concentrations ranging between 0.5 and 2.5 $gL^{-1}$. Alkylation reactions were monitored by HPLC until no further decrease in either **5** or **7** could be detected, at which point reactions were quenched. For identification of alkylated products, appropriate fractions were collected from the analytical column and analysed by MS. It should be noted that octylamine was a major interference in CI/ EIMS. Retention factors for **5** and **7** were 2.1 and 3.8 respectively which was acceptable for monitoring purposes. Alkylated products of **5** and **7** exhibited greater retention factors.

### *7.4.4 Gas Chromatograph Instrumentation*

The GCMS system was described in Section 7.1. The analytical column used was HP-1 (25m x 0.32mm i.d., 0.52μm film thickness). The GC temperature program was as follows: initial temperature 50°C held for 1 min.; increased to 150°C at 30°C/ min, then to 290°C at 10°C/ min, held for 2 min. The injector temperature was 260°C and detector 290°C. Samples (1μL) were injected in the split mode (10:1). The mass spectrometer was operated at 70eV with electron impact ionisation using the scan mode.

### *7.4.5 Preparation and Analysis of Reaction Solutions by GC*

Ester samples taken from alcoholic reaction solutions were quenched (usually by pH adjustment), dried, placed in ampoules then diluted to about 1mL with $CHCl_3$ and analysed.

Ester samples ex silica work-up (5-10mg) were placed in ampoules, dissolved in about 1 mL $CHCl_3$, sealed then injected onto the chromatograph.

## 7.5 Organic Preparations

Known methods were used, usually with some modification, to produce **5**,[2] **7**,[3-5] **10**,[6] **14**,[7] **15**,[6] **16**,[8] **17**,[8] **21**,[9] **23**,[10] **25**,[9] **26**[2] and **30**.[11] Compounds **1a**, **8**, **19**, **22**, **24** and **27** were prepared according to methods developed by the author.

Typical preparations are given and no attempts were made to optimise yields.

**2-Oxo-1-piperazineacetic acid (5)**

*N,N'*-Ethylenebis(aminomalonic) acid.1/3 $H_2O$ (**15**) (13.5 g, 50 mmol) was heated at reflux with 100 mL deionised $H_2O$ until cessation of $CO_2$ evolution. Concentrated HCl (32%, 1 mL) was added and the solution heated at reflux for a further 3 h. After cooling slightly the solution was evaporated to dryness, recrystallising the crude product twice in MeOH/$(CH_3)_2CO$ to obtain the monohydrochloride of **5** in 69.8% yield.

$^1$H NMR ($D_2O$, δ ppm) 4.21 (s, 2H), 3.95 (s, 2H), 3.73 (t, 2H, J = 6 Hz), 3.59 (t, 2H, J = 6 Hz); $^{13}$C NMR 171.6, 165.4, 54.1, 49.8, 45.6, 41.3; LSIMS m/z: 159 ($MH^+$) Calc. for $C_6H_{11}N_2O_3$ 159.0770, found 159.0776; Anal. Calc. for $C_6H_{10}N_2O_3$.HCl: C, 36.99; H, 5.65; N, 14.38. Found C, 36.95; H, 5.75; N, 14.30.
The dihydrochloride of ethylenediamine-*N,N'*-diacetic acid (**10**) was recovered from the filtrate in less than 3% yield.

**Piperazinone (7)**

Ethyl chloroacetate (9.53 g, 78 mmol) in 50 mL dry EtOH was added dropwise over 2 h to a stirred solution of ethylenediamine (30.2 g, 5000 mmol) in 100 mL dry EtOH. After standing at room temperature for 2 h solvent and excess ethylenediamine were removed under vacuum and the crude product neutralised with alcoholic KOH (4.42 g/ 25 mL). The resulting precipitate of KCl was removed by filtration and the yellow filtrate concentrated under vacuum. The oily residue was distilled in a Kugelröhr apparatus (160°C / 1mm Hg) to furnish pure white crystals of **7** in 35.8% yield.

$^1$H NMR ($D_2O$, δ ppm) 3.33 (s, 2H), 3.28 (t, 2H, J = 5.3 Hz), 2.87 (t, 2H, J = 5.3 Hz); $^{13}$C NMR 173.7, 48.3, 42.4, 41.3; UV ($\lambda_{max}$ 214nm, MeCN/ $H_2O$ 1:1) ε= 1331; EIMS m/z: 100 ($M^+$, 100%; Calc. for $C_4H_8N_2O$ 100.0636, found 100.0636), 71(45), 43(70);

Anal. Calc. for $C_4H_8N_2O$: C, 47.98; H, 8.07; N, 27.97. Found C, 48.14; H, 8.40; N, 28.21.

**4-(2-Aminoethyl)-piperazinone (8)**

To a stirred aqueous solution of **7** (0.50 g, 5 mmol) at 70-80°C was added dropwise 2-chloroethylamine HCl (0.70 g, 6 mmol) in 30 mL deionised water. The pH was maintained between 8-9.5 with solid portions of $Na_2CO_3$. Samples taken at regular intervals and analysed by HPLC showed the reaction to be "complete" after about 4 h. The fraction eluting at k ~ 4.9 was collected from the HPLC column (HPLC conditions section 7.4.1) and found to be consistent with **8** (high resolution MS). After removing $H_2O$ under reduced pressure several attempts were made to isolate **8** from the reaction mixture but little success was achieved. The quantity of **7** was reduced by simple extractions with dry EtOH but removal of excess 2-chloroethylamine proved difficult. No other analytical data were obtained for **8**.

EIMS m/z: 143 ($M^+$, 22%; Calc. for $C_6H_{13}N_3O$ 143.1060, found 143.1061), 113(100), 85(47), 56(20)

**Bromomalonic acid, disodium salt (14)**

Bromine (45 mL, 870 mmol) dissolved in approximately 225 mL of $CCl_4$ was added dropwise to a stirred solution of powdered malonic acid (90.9 g, 870 mmol) dissolved in approximately 1800 mL $Et_2O$ on ice. At the end of the reaction the bulk volume of solvent was removed by gentle distillation. The final 100 mL or so was removed on a vacuum line at ambient temperature. The crude product was dissolved with a minimum quantity of deionised $H_2O$ and the pH adjusted to 8.5 with 30% NaOH (on ice) to give **14**. The solution containing **14** was then poured into approximately 3L of EtOH. The white product (yield 69.0%) was collected by filtration, dried on a vacuum line and used without further purification.

$^1$H NMR ($D_2O$, δ ppm) 4.65 (s, 1H) ; $^{13}$C NMR 176.9, 55.8, 55.4; LSIMS m/z: 182.9 ($MH^+$) Calc. for $C_3H_4BrO_4$ 182.9293, found 182.9285; Anal. Calc. for $C_3HBrO_4.Na_2$: C, 15.87; H, 0.44. Found C, 15.82; H, 0.68.

### *N,N'*-Ethylenebis(aminomalonic) acid (15)

Bromomalonic acid, disodium salt (56.8 g, 250 mmol) was dissolved in approximately 180 mL deionised $H_2O$ with a catalytic amount of KI and the pH adjusted to about 9 with 30% NaOH. To this stirred solution was added ethylenediamine (7.6 g, 130 mmol) and the mixture was heated at 70°C for 2.5 h, maintaining pH 9-11 with 30% NaOH. On cooling, the solution was adjusted to pH 1-2 with conc. HCl (32%) on ice and the resulting precipitate collected by filtration and washed with deionised $H_2O$ and MeOH. For purification crude **15** was dissolved in aqueous alkali, precipitated with conc. HCl then collected by filtration, washing with small volumes of cold $H_2O$ and MeOH. The purification procedure was repeated three times, furnishing *N,N'*-ethylenebis(aminomalonic) acid.1/3 $H_2O$ in 48.8% yield.

Anal. Calc. for $C_8H_{12}N_2O_8$.1/3 $H_2O$: C, 35.55; H, 4.69; N, 10.37. Found C, 35.45; H, 4.61; N, 10.42.

**Note :** several attempts were made to obtain $^1$H/$^{13}$C NMR spectra for **15** but poor solubility in deuterated solvents prevented acceptable analyses.

### 2-Chloroethyliminodiacetonitrile (16)

2-Chloroethylamine HCl (120.2 g, 1040 mmol) was placed with 200 mL deionised $H_2O$ in a 1L flask equipped with condenser and paddle stirrer. A 35% solution of formaldehyde (177.9 g, 2070 mmol) was added dropwise to the stirred, cooled (0-5°C) solution of 2-chloroethylamine HCl, followed by conc. HCl (32%, 98 mL, 1030 mmol) over a 30 min period. A 35% solution of KCN (134.9 g, 2070 mmol) was then added dropwise to the reaction solution over a period of 3-4 h. The reaction solution was stirred for up to 20 h before collecting the white precipitate by filtration, washing

with small volumes of cold $H_2O$ and EtOH. The white product (**16**) was partially dried under vacuum then recrystallised a single time from EtOH (yield 75.5%).

$^1H$ NMR (DMSO, δ ppm) 3.92 (s, 4H), 3.75 (t, 2H, J = 6.4 Hz), 2.93 (t, 2H, J = 6.4 Hz); $^{13}C$ NMR 115.7, 53.9, 41.8, 41.0; EIMS m/z: 157 ($M^+$, 25%; Calc. for $C_6H_8ClN_3$ 157.0407, found 157.0410), 131(18), 108(100), 67(23); Anal. Calc. for $C_6H_8ClN_3$: C, 45.66; H, 5.07; N, 26.63. Found C, 45.53; H, 5.11; N, 26.51.

**2-Chloroethyliminodiacetate, dimethyl (17)**

2-Chloroethyliminodiacetonitrile (10.23 g, 65 mmol) was placed with 80 mL dry MeOH and dissolved with heating and stirring. Dry HCl gas (generated from $H_2SO_4$/ $NH_4Cl$) was passed into this vigorously stirred solution at constant rate, at reflux, for about 4 h. After HCl addition the amber coloured solution was stirred for an additional 4 h before removing heat. The reaction solution was allowed to cool then stored at 0°C overnight.

The chilled solution was filtered and the filtrate evaporated to dryness. The pH of the residue was adjusted to 6 using a combination of ice $H_2O$ and 30% KOH, then to 8 with solid portions of $K_2CO_3$. The alkaline solution was extracted with 120 mL (3 x 40 mL) $Et_2O$, the ether extracts dried over $Na_2SO_4$ then decolourised with a small quantity of activated carbon. After removing the carbon (filtration) and solvent, crude **17** was purified by silica gel chromatography, ($CHCl_3$:EtOH 99:1). No further purification was required and **17** was obtained in 33.0% yield.

$^1H$ NMR ($CDCl_3$, δ ppm) 3.60 (s, 6H), 3.52 (s, 4H), 3.48 (t, 2H, J = 6.8 Hz), 3.00 (t, 2H, J = 6.8 Hz); $^{13}C$ NMR 171.5, 56.3, 55.2, 51.2, 42.0; EIMS m/z: 223 ($M^+$, 5%; Calc. for $C_8H_{14}ClNO_4$ 223.0611, found 223.0605), 187(13), 164(100), 136(36), 106(23); Anal. Calc. for $C_8H_{14}ClNO_4$: C, 42.95; H, 6.32; N, 6.26. Found C, 43.06; H, 6.30; N, 6.36.

**4-[2-[bis(Carboxymethyl)amino]ethyl]-2-oxopiperazine (19)**

A stirred solution of **7** (3.50 g, 35 mmol) and **17** (7.5 g, 34 mmol) in 50 mL dry MeOH was heated at 65°C for 10 min before dropwise addition of $Et_3N$ (5.5 g, 54 mmol). After stirring for an additional 6.5 h excess $Et_3N$ and MeOH were removed under vacuum and the residue stored at 0°C overnight.

Dry $(CH_3)_2CO$ (10 mL) was added to the residue and the resultant precipitate ($Et_3N$.HCl with some piperazinone HCl) was removed by filtration. After evaporation of the $(CH_3)_2CO$ the crude residue was reconstituted in a small volume of $H_2O$ and extracted with 60 mL (2 x 30 mL) of $Et_2O$. The aqueous phase was evaporated to dryness, dissolved in dry $CH_2Cl_2$ then filtered. The volume of the filtrate was reduced almost to dryness and the residue purified by silica gel chromatography ($CH_2Cl_2$:EtOH 95:5) giving **19** (yield 31.1%) as a light yellow oil. $^1H$/ $^{13}C$ NMR are shown in Figure 7.1.

$^1H$ NMR ($CDCl_3$, δ ppm) 6.82 (J = 2.1 Hz), 4.75 (s, 6H), 3.54 (s, 4H), 3.31 (m, J = 5.6 Hz, J = 2.1 Hz), 3.14 (s, 2H), 2.86 (t, 2H, J = 6.4 Hz), 2.69 (t, 2H, J = 5.6 Hz), 2.58 (t, 2H, J = 6.4 Hz); $^{13}C$ NMR 171.6 (<u>C</u>OOMe) 169.2 (NH<u>C</u>O$CH_2$) 56.8 (NHCO<u>C</u>$H_2$) 55.8 [<u>C</u>$H_2$N($CH_2$COOMe)$_2$] 55.4 (<u>C</u>$H_2$COOMe)$_2$ 51.5 (OMe) 50.7 (NHCO$CH_2$N<u>C</u>$H_2$) 49.0 (NH$CH_2$<u>C</u>$H_2$N) 41.0 (NH<u>C</u>$H_2CH_2$N); FTIR (KBr disc) 1750, 1675cm$^{-1}$; LSIMS m/z: 288 ($MH^+$) Calc. for $C_{12}H_{22}N_3O_5$ 288.1560, found 288.1576; Anal. Calc. for $C_{12}H_{21}N_3O_5$: C, 50.16; H, 7.38; N, 14.62. Found C, 48.79; H, 7.43; N, 14.15. **Note :** sample contained residual $CH_2Cl_2$, ~1/7 mole, which extremely difficult to remove. $CH_2Cl_2$ confirmed by GCMS).

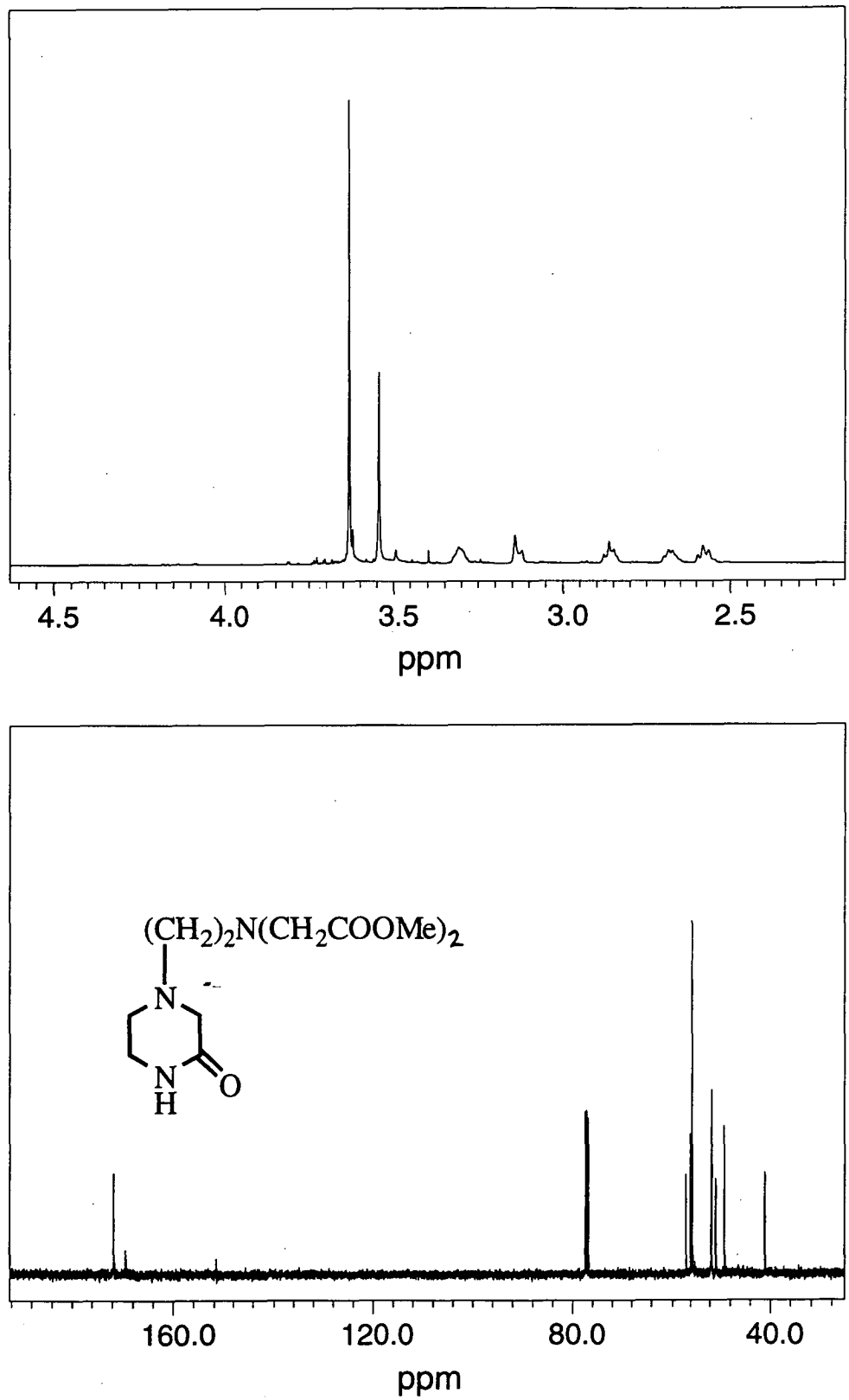


*Figure 7.1* $^{1}H$ *and* $^{13}C$ *NMR spectra of compound* ***19***

**1-(Carboxymethyl)-4-[2-(carboxymethyl)aminoethyl]-2-oxopiperazine (22)**

A mixture of **25** (8.5 g) and **14** (21.1 g, 93 mmol) in 80 mL deionised $H_2O$ was stirred at 70-80°C for 2 h, maintaining pH 9-11 with 30% NaOH. After concentrating the solution to about 20 mL under vacuum, the pH was adjusted to 2 with conc. HCl and the solution heated at reflux until cessation of $CO_2$ evolution. The solution was then treated with additional conc. HCl and heated at reflux for another 1h. The solution was decolourised with activated carbon whilst still warm, filtered and evaporated to dryness. HPLC analysis of the residue showed the presence of at least four compounds and the desired molecular ion for $C_{10}H_{19}N_3O_5$ ($MH^+$) was identified by high resolution LSIMS. No further work-up of the residues was performed.

LSIMS m/z: 260 ($MH^+$) Calc. for $C_{10}H_{19}N_3O_5$ 260.1259, found 260.1261.

**bis(Salicylidineiminate)diethylenetriamine (23)**

Diethylenetriamine (60.0 g, 581 mmol) was added dropwise to a chilled and stirred (paddle stirrer) solution of salicylaldehyde (142.0 g, 1163 mmol) in 700 mL dry EtOH. After addition of diethylenetriamine the solution was stirred at room temperature for a further 5 h then EtOH removed under reduced pressure. Evaporation of solvent furnished **23** as a yellow oil. On standing in a desiccator large clear crystals of **23** (yield ca. 100%) formed.

$^1H$ NMR ($CDCl_3$, δ ppm) : 7.26 (t, 2H, J = 7.5 Hz), 6.79 (t, 2H, J = 7.5 Hz), 2.51 (t, 2H, J = 6.2 Hz), 1.77 (t, 2H, J = 6.2 Hz); $^{13}C$ NMR ($CDCl_3$, δ ppm) 163.2, 133.4, 119.7, 119.0, 117.9, 58.0, 50.6; LSIMS m/z: 312 ($MH^+$) Calc. for $C_{18}H_{22}N_3O_2$ 312.1707, found 312.1706; Anal. Calc. for $C_{12}H_{21}N_3O_2$: C, 69.43; H, 6.81; N, 13.49. Found C, 69.21; H, 6.82; N, 13.59.

**Ethyl bis(salicylidineiminate)-4-diethylenetriamineacetate (24)**

A solution containing **23** (5.0 g, 16 mmol), ethyl chloroacetate (3.1 g, 25 mmol) and $Na_2CO_3$ (1.7 g, 16 mmol) in 70 mL dry EtOH was heated at reflux for 4.5 h. After cooling slightly the reaction solution was concentrated to about 20 mL, filtered to remove salts then the filtrate evaporated to dryness. The crude residue was reconstituted in a minimum quantity of dry $CH_2Cl_2$ and the remaining insoluble inorganic salts removed by filtration. The $CH_2Cl_2$ was removed to give **24** (yield 72.7%) as a viscous yellow oil.

LSIMS m/z: 398 ($MH^+$) Calc. for $C_{22}H_{28}N_3O_4$ 398.2071, found 398.2070; Anal. Calc. for $C_{22}H_{27}N_3O_4$: C, 66.42; H, 6.79; N, 10.57. Found C, 66.20; H, 6.91; N, 10.84.

**4-Diethylenetriamineacetic acid (25)**

A solution of **24** (4.0 g, 10 mmol) in chilled 3M HCl (20 mL, 60 mmol) was stirred overnight then extracted with 80 mL (2 x 40 mL) dry $CH_2Cl_2$ to remove liberated salicylaldehyde. The aqueous phase was concentrated, the pH adjusted ca. 9 to with solid portions of $Na_2CO_3$, then the solution evaporated to dryness. The residues were reconstituted in dry MeOH, filtering to remove inorganic salts. The product, assumed to be the sodium salt of **25**, was used without further purification.

LSIMS m/z: 162 ($MH^+$) Calc. for $C_6H_{16}N_3O_2$ 162.1243, found 162.1255

**1-Methoxycarbonylmethyl-2-oxopiperazine (26)**

**a)**

Ethylenediamine-*N,N'*-diacetic acid (10.0 g, 58 mmol) was placed with 200 mL dry MeOH containing $SOCl_2$ (11.5 mL, 160 mmol) and heated at reflux for about 37 h. After cooling slightly, the solution was concentrated to 20-30 mL by evaporation, whereupon pure *N,N'*-bis(methoxycarbonylmethyl)ethylenediamine dihydrochloride

(yield 17%) separated from solution. This solution was stored overnight at 0°C then filtered. Immediately following filtration prolific crystallisation of the monohydrochloride of **26** occurred. The crystals were harvested by filtration (yield 57.6%) and no further purification was necessary.

The free base (**26**) was obtained in 91.0% yield by treating the monohydrochloride with one equivalent of NaOMe, removing solvent then dissolving **26** in $CH_2Cl_2$ to remove NaCl.

**b)**

**5** (7.0 g, 44 mmol) was placed with 150 mL dry MeOH containing $SOCl_2$ (8.0 mL, 112 mmol) and heated at reflux for 16 h. Work-up as per part a), yield 62.6%.

$^1$H NMR ($CDCl_3$, δ ppm) 3.92/ 3.90 (s, 2H, chair/ boat conformers), 3.41 (s, 2H), 3.40 (t, 2H, J = 5.6 Hz), 3.34 (s, 3H), 3.06 (t, 2H, J = 5.6 Hz); $^{13}$C NMR 175.6, 170.4, 51.7, 49.7, 49.6, 42.9; EIMS m/z: 172 ($M^+$, 74%; Calc. for $C_7H_{12}N_2O_3$ 172.0848, found 172.0853), 102(40), 85(78), 56(100); Anal. Calc. for $C_7H_{12}N_2O_3$.HCl: C, 40.28; H, 6.29; N, 13.43. Found C, 40.25; H, 6.28; N, 13.44; Anal. Calc. for $C_7H_{12}N_2O_3$: C, 48.84; H, 6.98; N, 16.28. Found C, 48.60; H, 6.94; N, 16.08.

**1-(Methoxycarbonylmethyl)-4-[2-[bis(methoxycarbonylmethyl)amino]ethyl]-2-oxopiperazine (27)**

To a stirred solution of **26** (9.6 g, 56 mmol) in 150 mL dry MeOH was added **17** (12.6g, 56 mmol). The solution was then heated to 65°C and after about 20 min $Et_3N$ (8.5 g, 84 mmol) added dropwise. After a further 11 h stirring at 65°C the solution was cooled slightly before removing the solvent under reduced pressure.

Dry $(CH_3)_2CO$ (40 mL) was added to the residue and the resultant white precipitate ($Et_3N.HCl$) was removed by filtration. After stirring the filtrate with a few spatula tips of activated carbon to decolourise, the solution was filtered once again and the filtrate evaporated to dryness. The amber coloured residue was dissolved in a minimum volume of deionised $H_2O$ then extracted first with 120 mL (3 x 40 mL) of $Et_2O$/ pet ether (4:1) then 120 mL (3 x 40 mL) $CHCl_3$. The $CHCl_3$ extracts were dried over $Na_2SO_4$, the $CHCl_3$ removed under reduced pressure then crude **27** purified by silica gel chromatography ($CHCl_3$:EtOH 99:1) giving pure **27** (yield 50.0%) as an amber coloured oil. $^1H/^{13}C$ NMR are shown in Figures 7.2.

$^1H$ NMR ($CDCl_3$, δ ppm) 4.08 (s, 2H), 3.68 (s, 3H), 3.64 (s, 6H), 3.55 (s, 4H), 3.41 (t, 2H, J=4.8 Hz), 3.33 (s, 2H), 2.95 (t, 4H, J=4.8 Hz), 2.68 (t, 2H, J=4.8 Hz); $^{13}C$ NMR δ: 171.49 [($\underline{C}OOCH_3)_2$], 169.02 ($\underline{C}OOCH_3$), 166.14 (NCO), 56.33 ($NCO\underline{C}H_2N$), 55.32 [($\underline{C}H_2COOCH_3)_2$], 55.25 [$NCOCH_2N\underline{C}H_2$)], 52.21 ($COO\underline{C}H_3$), 51.59 [($COO\underline{C}H_3)_2$], 50.52 [$\underline{C}H_2N(CH_2COOH)_2$], 49.31 ($\underline{C}H_2CH_2NCO$), 47.36 ($N\underline{C}H_2COOCH_3$), 46.54 ($CH_2\underline{C}H_2NCO$); UV ($\lambda_{max}$ 232nm, MeCN) ε= 790; FTIR (thin film) 1749, 1657$cm^{-1}$; EIMS m/z: 359 ($M^+$, 31%; Calc. for $C_{15}H_{25}N_3O_7$ 359.1692, found 359.1704), 300(22), 185(90), 174(100), 157(76), 146(50); Anal. Calc. for $C_{15}H_{25}N_3O_7$: C, 50.13; H, 7.03; N, 11.69. Found C, 48.11; H, 6.85; N, 11.31.

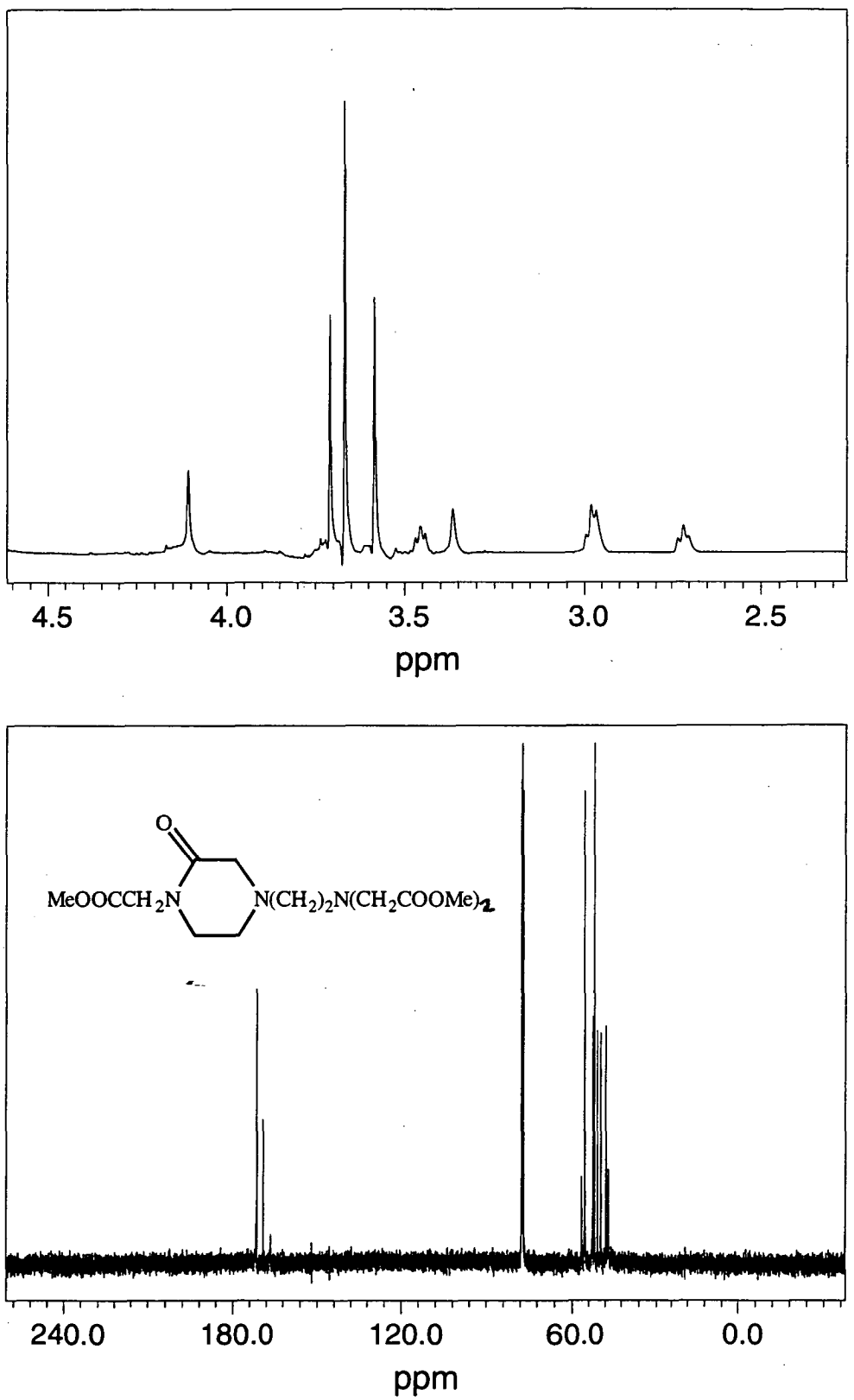


*Figure 7.2* $^{1}H$ *and* $^{13}C$ *NMR spectra of compound* ***27***

**1-(Carboxymethyl)-4-[2-[bis(carboxymethyl)amino]ethyl]-2-oxopiperazine (1a)**

**a)** from **19**

A solution of **19** (1.0 g, 3.5 mmol) and NaH (0.17 g, 7.4 mmol) in 25 mL dry THF was stirred at room temperature for 1 h then 80°C for 3 h. After cooling to room temperature, ethyl chloroacetate (0.55 g, 4.5 mmol) was added dropwise; the mixture was stirred 3 h at room temperature then 80°C for 5 h. The mixture was filtered, washing the solid with dry THF. After removal of THF from the filtrate, GCMS showed the residue to comprise mainly unreacted **19** with some **30**. Further treatment of the residue was not pursued.

The solid was treated for about 30 min with dilute HCl then $H_2O$ removed by evaporation. Analysis of the residues by LSIMS showed they contained (amongst others) **1a** and a compound with formula weight 259 (not identified). The relative quantities of **1a** / impurity were not determined.

EIMS m/z: 373 (M+, 10%; Calc. for $C_{16}H_{27}N_3O_7$ 373.1857, found 373.1858), 287(18), 202(25), 174(100), 146(40)

**b)** from **22**

A solution of crude **22** (a mixture of **21** and **22**) in 60 mL deionised $H_2O$ was neutralised with $Na_2CO_3$ before treating with an excess of sodium chloroacetate. This mixture was heated at 70-80°C for about 5 h, maintaining pH 8-9 with solid portions of $Na_2CO_3$. After standing overnight at 0°C the pH of the solution was reduced to 2 with conc. HCl and excess chloroacetic acid removed by extraction with $CH_2Cl_2$. HPLC of the crude product showed it to be a mixture of several amino acids including **21**, **22** and **1a** (Figure 4.9). Positive identification of **1a** was made by high resolution LSIMS. No further treatment (ie work-up) was performed on the crude reaction mixture.

c) from **27**

A solution of **27** (8.4 g, 23 mmol) in 80 mL deionised $H_2O$ containing 3 mL conc. HCl was heated at reflux for about 3 h. The solution was decolourised with a small amount of activated carbon, filtered and the filtrate evaporated to dryness. The trihydrochloride of **1a** was recovered in 75.2% yield. Several unsuccessful attempts were made to crystallize the trihydrochloride salt. The trihydrochloride salt was hygroscopic and stored over desiccant when not in use.

$^1$H NMR ($D_2O$, δ ppm) 4.09 (s, 2H), 3.92 (s, 2H), 3.67 (t, 2H, J = 6 Hz), 3.56 (s, 4H), 3.54 (t, 2H, J = 6 Hz), 3.27 (t, 2H, J = 5.6 Hz), 3.08 (t, 2H, J = 5.6 Hz); $^{13}$C NMR 175.66 [$(CH_2\underline{C}OOH)_2$], 172.70 ($CH_2\underline{C}OOH$), 164.99 (NCO), 56.77 ($\underline{C}H_2COOH$), 55.51 ($NCOCH_2N\underline{C}H_2$), 53.90 ($NCO\underline{C}H_2N$) 50.32 [$\underline{C}H_2N(CH_2COOH)_2$], 49.58 ($\underline{C}H_2CH_2NCO$), 49.37 [$(\underline{C}H_2COOH)_2$], 49.17 [$(\underline{C}H_2COOH)_2$], 45.33 ($CH_2\underline{C}H_2NCO$). Exchangeable (acidic) protons were detected by $^1$H NMR (DMSO) at 100°C. A single broad exchangeable peak at 8.9δ gave an integration of 6H, consistent with the microanalysis. The position of the peak at 8.9δ was indicative of rapid exchange (NMR timescale) between ligand protons and free HCl protons (which occur at 5.2δ in DMSO); FTIR (KBr disc) 1739 and 1661cm$^{-1}$; LSIMS m/z: 318 ($MH^+$) Calc. for $C_{12}H_{20}N_3O_7$ 318.1301, found 318.1315; Anal. Calc. for $C_{12}H_{19}N_3O_7.3HCl$: C, 33.74; H, 5.21; N, 9.84. Found C, 33.34; H, 5.43; N, 9.69.

## 7.6 Removal of Impurity from Ligand 1a

After detecting a small percentage (~ 5%) of impurity in **1a** by $^1$H NMR titration (Chapter 6, section 6.6.1) steps were taken to remove it. Several approaches were attempted, some of which are now described.

### *7.6.1 Crystallisation of the Trihydrochloride Salt*

For many aminopolycarboxylic acids simple water-alcohol mixtures have been used to recrystallise.[2,6,12] Acetone has also been frequently used.[2,12] Such an approach could not be used in the given case due to the complete solubility of **1a** trihydrochloride in cold $H_2O$, MeOH and EtOH. Furthermore it had been shown (using LSIMS/EIMS) that partial reesterification of **1a** could occur during treatment with hot MeOH . The ligand as the trihydrochloride was found to be insoluble in hot chlorinated solvents, MeCN, EtOAc and THF. These solvents were thought most appropriate for recrystallisation and no other solvents were trialed. Several solvent mixtures, including MeOH/DCM, MeOH/$CHCl_3$ and MeCN/$Et_2O$ were used without success; the main disadvantage using these mixtures was the presence of MeOH.

Evaporation of water from an aqueous solution containing **1a** trihydrochloride using a slow, steady stream of nitrogen caused "clumps" of product to form at the interface. Individual crystal growth was not observed. Similar results were obtained when EtOH was used in place of water.

### *7.6.2 Crystallisation of the Free Acid*

Much of the difficulty associated with recrystallisation of the trihydrochloride salt has been attributed to its hygroscopic nature. Similar difficulties were encountered by other authors[9,13] when trying to recrystallise salts of aminopolycarboxylic acids. Whilst it was convenient and practical to preserve **1a** as a trihydrochloride salt for titrations, in order to address the impurity situation attempts were made to generate the free acid form for further recrystallisation trials. The method used to produce free

**1a** involved treatment of the trihydrochloride with the calculated amount of either weak inorganic (eg. $NaHCO_3$) or weak organic base (eg. $Et_3N$). The free acid was readily formed in these ways but cleaning up inorganic/organic salts proved extremely difficult. For example, organic bases (eg. $Et_3N$) effectively buffered the hydrochloride but subsequent separation of $Et_3N.HCl$ from free **1a** was not achieved. Extraction of an aqueous solution containing **1a** and $Et_3N.HCl$ with $CHCl_3$ resulted in both **1a** and $Et_3N.HCl$ being extracted into the organic layer. Further workup of the extract failed to yield the free acid form of **1a**.

### *7.6.3 Other Purification Procedures*

As indicated in section 7.5 bromomalonic acid and *N,N'*-ethylenebis(aminomalonic) acid were purified / isolated as sodium salts. This procedure was also pursued for **1a**. Whilst there was little difficulty producing quantities of the trisodium salt, either by treatment with $Na_2CO_3$ or $NaHCO_3$, inorganic salts were difficult to eliminate. Like the trihydrochloride, the trisodium salt proved soluble in MeOH and EtOH.

One option for preparation of the free acid, elution of the trisodium salt through catex resin, proved unsuccessful. The ligand could not be recovered from the column despite copious washing with $H_2O$ and various alcohol-water, acetonitrile-water mixtures. Agitating the loaded resin for several hours in water also failed to liberate the free acid. A similar result had been observed previously for the HCl salt of 4-diethylenetriamineacetic acid and other derivatives of this acid.

Attempts made to freeze out the trihydrochloride from various aqueous solutions also failed. Consideration was also given to purification by complexation but a suitable technique could not be located or developed.

## 7.7 Conclusions of purification work

As indicated some considerable time and energy were put toward purification of **1a** without reward. A considerable portion of ligand **1a** was consumed by the purification work and could not be recovered. A decision was made to continue with the titration work bearing in mind **1a** contained a small percentage of impurity (< 5%) and that this impurity could have a negative influence on potentiometric titrations.

## 7.8 Experimental for Chapter Six

### *7.8.1 NMR Determinations*

Natural abundance spectra were recorded using a Varian Inova-400 Wide Bore instrument with an inverse detection probe operating at 399.98 ($^{1}$H) and 100.58 ($^{13}$C) MHz. Titrations were performed in water containing ligand, background electrolyte (sodium perchlorate) and/or metal perchlorate. Calibration and lock were achieved by means of a capillary insert containing benzene-$d_6$ and TMS. Water resonance suppression was implemented via a binomial 1-3-3-1 presaturation pulse sequence or by shaped pulses. Peak assignments during the course of titrations were made possible by the use of a gradient enhanced COSY pulse sequence; presaturation of the water resonance was unnecessary in this case. NMR analysis of an aqueous solution containing only background electrolyte showed no organic signals other than residual protonated benzene and TMS.

Up to 20 $^{1}$H spectra were recorded between pH 2-11 and TMS calibration was used with every experiment.

## 7.9 References

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# CHAPTER 8

## Conclusions

Identifying the processes that are most likely to contribute to the degradation of DTPA in pulping liquors (Chapter 2) provided valuable insight into possible ways of synthesising the cyclic DTPA degradation product 1-(carboxymethyl)-4-[2-[bis(carboxymethyl)amino]ethyl]-2-oxopiperazine (**1a**). The similarity between breakdown products of both EDTA and DTPA due to these processes (chemical oxidation, photodegradation and biodegradation) strongly implicated a universal mode of decomposition, namely *successive oxidative decarboxylation.* This mechanism has been verified in vitro using the action of $KMnO_4$ on DTPA in acidic aqueous conditions.[1] Furthermore, in each of the three methods developed for manufacture of ligand **1a**, oxidative decarboxylation was utilised.

As described in Chapter 4 it was possible to produce **1a** by three individual but related methods. It was necessary to fully develop one of these only (Scheme II′, section 4.2). Sufficient amounts of **1a** (as the trihydrochloride) were produced using Scheme II′ for subsequent complexing studies.

Of the techniques available for determination of equilibrium constants, NMR and potentiometry were selected for this study. The protonation constants determined by $^1H$ NMR and potentiometric titrations showed general agreement (section 6.9) and compared favourably with published values for **1b**. The stability sequence obtained was Fe(III) > Zn(II) > Hg(II) ≅ Pb(II) > Mn(II) and $\log K_{ML}$ values ranged between 12.5 and 16.5. Immediately obvious from these data is the significant decrease (as expected) in the stability of **1a** metal complexes compared with DTPA metal complexes. However it is clear that **1a** possesses quite considerable complexing

ability, comparable to NTA for Hg(II) and Fe(III) complexes and EDTA for Zn(II), Pb(II) and Mn(II) complexes (Chapter 6, Table 6.5).

The stability sequence is also slightly different to what might be anticipated in light of data from similar ligands. However, the least stable ($Mn^{2+}$) and most stable ($Fe^{3+}$) complexes are in agreement with previous published rankings.[2] The range of log stability constants is also much narrower than the corresponding range for DTPA. One could have expected a much greater difference between the divalent metals and trivalent Fe. Such a difference occurs for EDTA and DTPA, less so for NTA. Perhaps keen competition (from water) for coordination sites about ligand **1a** prevents Fe(III) from forming stronger chelates compared to the given divalent metal ions. Without further investigation (eg. molecular mechanics/ X-ray analysis) it is difficult to assign a definitive reason for the apparent low stability constant value for Fe(III).

The major outcome of this study is that the use of DTPA as a chelating agent in thermomechanical pulping at ANM (Albury) is appropriate given the significant reduction in complexing power and concentration of DTPA degradation products such as **1a** in pulping liquors. Process changes with respect to DTPA (eg. dosage) would appear to be unnecessary. The other major outcome of this investigation is that a monitoring program for **1a** may need to be implemented (section 8.1).

## 8.1 Future research

The next obvious step would be to determine actual levels of **1a** in pulping liquors. It has been demonstrated previously that **1a** can be detected as its ferric complex by HPLC in the same manner as DTPA.[1] Thus an analytical method already exists and it should be possible to use remaining **1a** from this study as a standard for determinations. The level of **1a** in pulping effluent is expected to be less than DTPA[3] (ie < 5 ppm) and thus should pose no major concerns whether effluent is discharged to land or catchments.

Other possibilities for further investigation might include :

- the use of **1a** as a chelating agent (or precursor) in its own right. It has been found that the cyclic DTPA degradation product has considerable complexing ability and so could prove to be a useful ligand or ligand precursor

- X-ray analysis of **1a** crystals and **1a** complexes to gain more information about the likely form and solution behaviour of the ligand and its complexes. Molecular mechanics may also prove useful in such an examination

- identification of the specific locations of DTPA degradation in the papermaking process. Whilst this would be useful in terms of helping complete the file on **1a** it may not offer much more practical benefit, since the information would not really have any process implications

There do not appear to be many other lines of investigation (in terms of the papermaking process) involving **1a**.

## 8.2 References

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